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PHYTOPATHOLOGY

VOLUME 23 NUMBER 3

MARCH, 1933

THE PINEAPPLE MEALY BUG, PSEUDOCOCCUS BREVIPES, AND WILT OF PINEAPPLES[1]

WALTER CARTER

(Accepted for Publication June 29, 1932)

INTRODUCTION

The first reference to wilt of pineapples in Hawaii was made by Larsen (15) in 1910. From his description and photographs it is evident that the condition was present in the islands at that time. Higgins (11, p. 30), in 1912, reported damage from wilt as limited to a few fields. Since the organization of experimental work on pineapples, numerous references to plant failure have been made in local records under the generic term "wilt." The problem evidently increased in importance, for by 1920, according to Illingworth (13), "whole fields were wiped out." By the end of the next decade, the wilt problem was recognized as the most serious with which growers had to deal.

It is evident from a consideration of the literature that the term wilt has been used in a generic sense to include many types of plant failure. Sideris and Paxton (17) have summarized the literature on root-rotting fungi with respect to plant failure and concluded that "Root rot caused by pythiaceous organisms is probably one of the important causes for the development of the disease of pineapple plants known as 'wilt' ". These same authors (16) also report pythiaceous organisms of heart rot as contributing to plant collapse under extremely localized and specific conditions of temperature and humidity. Their conclusions, however, as to the effect of root rot on the development of wilt are as follows: "Resistance in pineapples may be interpreted as the ability of infected plants to produce new roots and thus perpetuate the life of the plant and susceptibility as the converse of this reaction."

Illingworth (13) presented the first evidence that the pineapple mealy bug, *Pseudococcus brevipes* (Ckl.), was an important factor and concluded that wilt was a disease transmitted by mealy bugs that had fed upon diseased plants. He also considered that the green spotting of the leaves, which sometimes follows mealy-bug feeding, was a characteristic symptom

[1] Published with the approval of the Director as Technical Paper No. 47 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

of wilt that could be inherited from mother plants by the vegetative planting material.

In the present writer's investigations, described in this paper, the first step was to determine conclusively the relationship between *Pseudococcus brevipes* and pineapple wilt. In the course of such study other significant questions arose that are related to the principal problem. These were duly considered and the results are included in this paper. A concurrent study of the various types of spotting caused by mealy-bug feeding has been conducted and the results are being published elsewhere (5).

DESCRIPTION OF THE DISEASE

"PINEAPPLE WILT": "EDGE WILT"

Larsen (15) described the most characteristic symptom as loss of rigidity of the leaves. Color changes were noted as occurring but not without exception. Roots were invariably found dead and filled with fungus mycelia. His illustration is typical of what is now recognized as mealy-bug wilt. Illingworth (13) also described the disease, calling attention to color changes associated with loss of rigidity. He also mentioned the fact that, typically, wilt begins at the edges of fields or rock piles within field areas.

FIG. 1. Aerial photograph of pineapple field, showing distribution of wilt along field margins and intra-field ditches. Photographed by the 11th Photo Section A. C., Luke Field, Oahu, Territory of Hawaii.

This edge wilt, as plantation men came to call it, appeared to the writer, after numerous field observations, as a most significant concept. It suggested strongly the invasion of the field by some causal agent. Figure 1 is an aerial photograph of a field showing edge wilt in pronounced degree.

FIG. 2. A. Quick wilt produced in field plots. B. Slow wilt occurring after large colonies of mealy bugs had fed on plants for approximately 1 year.

It will be observed that the lighter areas, which represent areas of wilting plants, are concentrated either on the field edges or next to weedy drainage ditches.

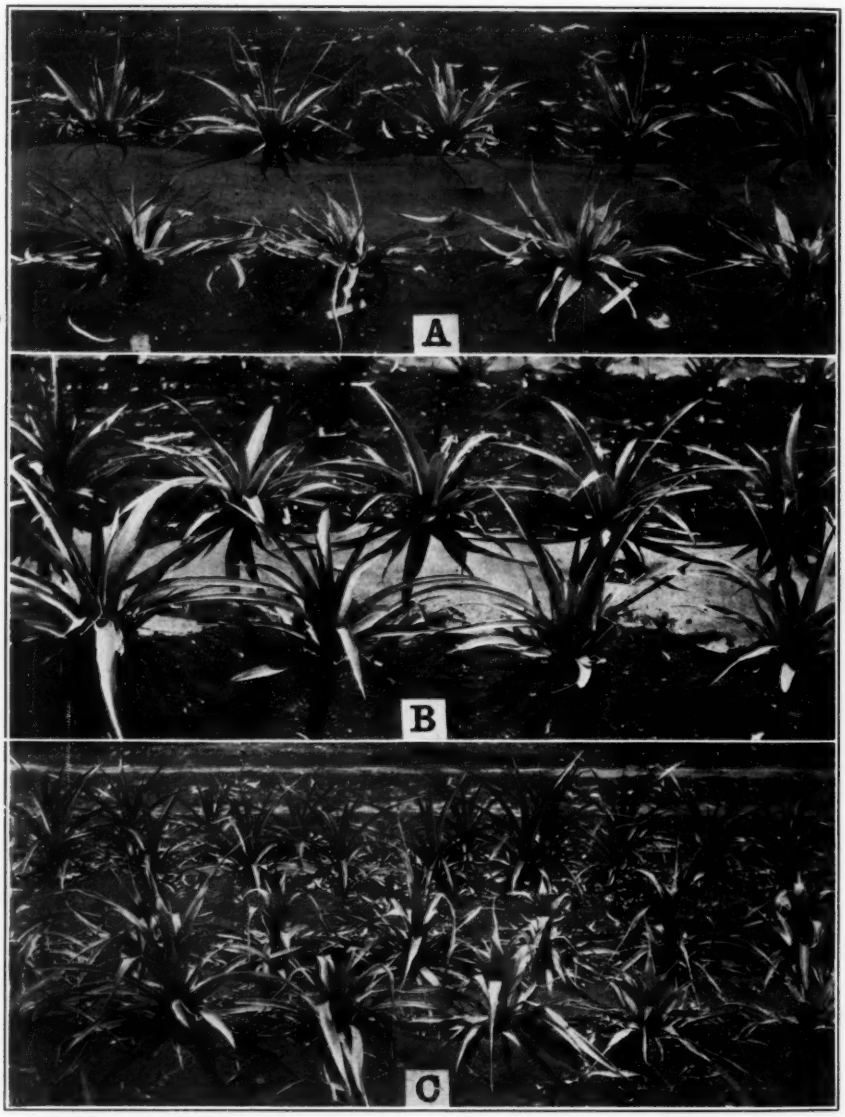

FIG. 3. A. Wilt produced by infesting each plant with 50 mealy bugs on February 3, 1931. Photographed April 30, 1931. B. Check plants of figure 3, A. C. In foreground plants infested with 50 mealy bugs per plant on April 27, 1931; in background check plot. Photographed July 6, 1931.

The following descriptions of the disease are based on plants used in experiments that are described in detail in this paper. In view of the terms used, the descriptions may be divided under the following headings:

QUICK WILT (FIGS. 2–5)

Occurs primarily in young plants. In plants up to 6 months old, the inner leaves become pale, varying from very light dull green to pale yellow

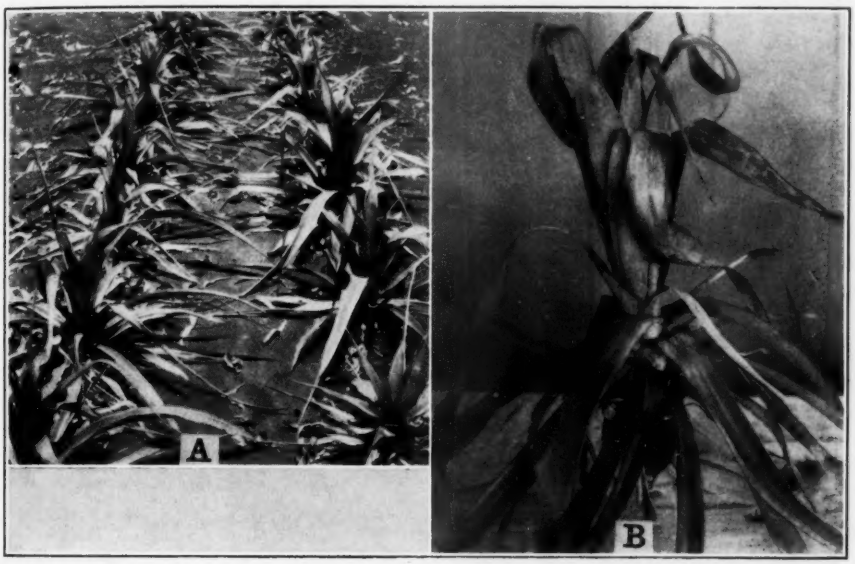

FIG. 4. A. Plot with quick wilt. B. Individual plant from plot shown in A. Photographed July 20, 1931.

or pink. With the color change is a very characteristic loss of rigidity, most of the inner leaves becoming flaccid and bending outward. The tips of these leaves turn brown and dry up. The inner leaves may show many or few or no green spots, or there may be scattered small chlorotic areas with irregular margins. With plants 8 to 10 months old, there is a conspicuous reddening of the leaves of the fourth or fifth whorl[2] from the center with a progression of color change to red, inward, to the center whorls. From that point color changes may occur either in the direction of bright pink and yellow or to a flat yellow brown. Development of the pink and red shade is controlled somewhat by the light-shade conditions. In dense plantings, the yellow and brown shades predominate. Secondary necrotic

[2] The term "whorl" is used for convenience in designating groups of 3 contiguous leaves in the normal spiral in which pineapple leaves grow.

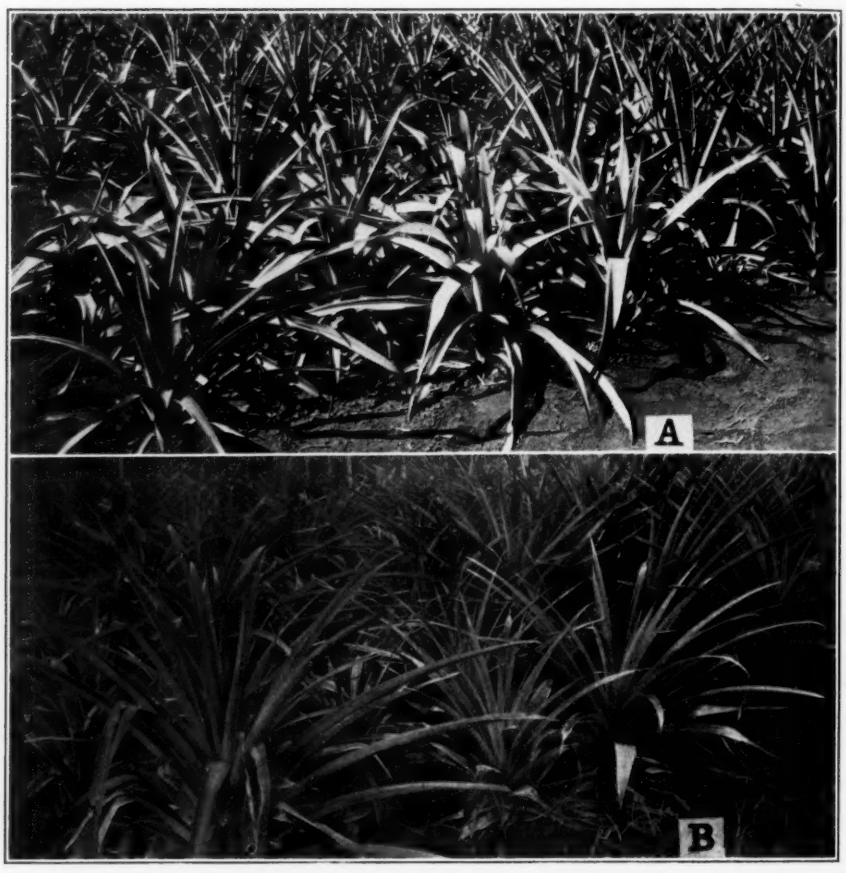

FIG. 5. A. Quick wilt in 4-line plot of Experiment III. Photographed July 20, 1931. B. Recovery in same plot. Photographed February 9, 1932.

areas appear on affected leaves, presumably the result of invasion of old feeding punctures by saprophytic organisms. When quick wilt occurs at early inflorescence or fruiting stages, the general appearance is one of dried up yellow brown leaves, frequently without the outward reflexing of the leaves. Quick wilt in young suckers and slips follows the same course as in very young mother plants. This type of wilt is produced by a short period of feeding by a fairly large colony of mealy bugs.

SLOW WILT

Wilt, occurring after the development of a large colony of mealy bugs, shows fewer color changes. A most characteristic symptom on slow-wilted plants is the large number of old mealy-bug feeding points. These may be of the green-spotting or chlorotic-spotting types (5). The leaves in this

type of wilt are usually so completely covered with these feeding areas as to eliminate most of the leaf tissue from functioning. The tips of these leaves are browned and dry and those of the outer leaves bend outward and droop, but there is none of the yellow pink characteristic of the quick-wilt type. The edges of the inner leaves are reflexed inward and flaccid to the touch but remain upright and retain a drab green color (Fig. 2, B). Secondary necrosis is commonly encountered.

In both types of wilt the roots are collapsed, invaded by saprophytic organisms, or dried up.

THE PINEAPPLE MEALY BUG

Pseudococcus brevipes is a member of the Homopterous family Coccidae. It has been reported (8) as partial to bromeliaceous and allied plants and is believed to be indigenous to Central and South America. In Hawaii it is found on a large number of host plants belonging to many families without close affinities. The principal hosts are pineapples, *Ananas comosus* (L.) Merr., banana, sisal, and several grass species, of which panicum grass, *Panicum barbinode,* and red-top Natal grass, *Tricholaena rosea* Nees, are the most important.

The insect is parthenogenetic and viviparous; its rate of development is slow, at least 2 months being required for development from newly emerged larva to gravid female; it is covered by a whitish secretion of wax and secretes honeydew; and it is almost invariably attended chiefly by two species of ants, *Pheidole megacephala* (Fabr.), and *Solenopsis geminata* Fabr. var. *rufa* Jerdon, respectively.

At the time of harvesting, the mealy bugs are found in large numbers on the fruit and on the slips and shoots that grow out from the fruit and mother stems. Damage to fruit has been recognized for some time (9) and its quantitative estimation determined recently (6).

The inception of infestation and its development and spread in pineapples have been studied and data presented in another paper (4). The movement into fields from wild hosts is characteristic and significant. Populations are thus established, first on the edge of fields with a gradual movement into the field area.

When these facts were established, the possible causal connection between edge wilt and the invasion of the field edges by mealy bugs was considered. Experimental work began with tests of *Pseudococcus brevipes* as a single factor in the production of wilt.

Experiment I. Pseudococcus brevipes as a single factor in the production of pineapple wilt

The planting material used for this experiment was obtained from a field that had just previously had its first or "plant-crop" fruit removed.

At this time the old fruit stem bears a number of "slips," which, when broken off and cured by drying, are used to plant new fields. The field from which these slips were taken was infested with mealy bugs and all the

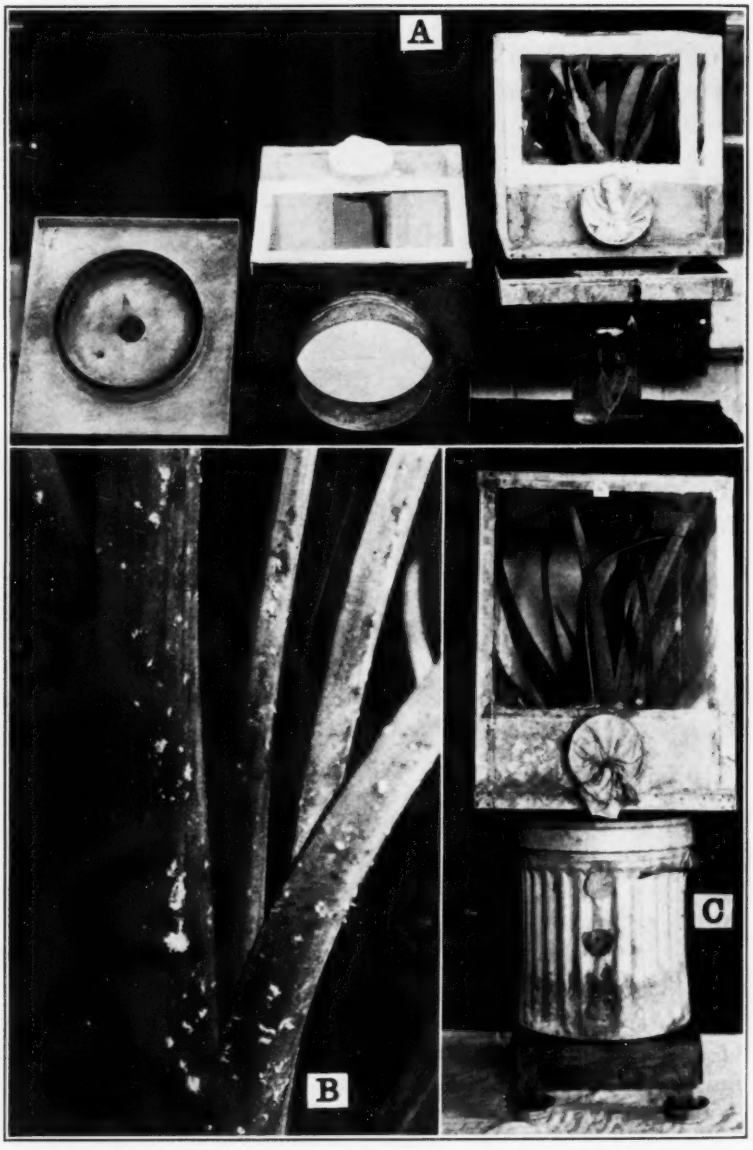

FIG. 6. A. Cage used for protecting pineapple plants growing in culture solution from accidental insect infestation. B. Mealy-bug colony unattended by ants. C. Cage used over pineapple plant growing in soil.

plants were green-spotted. Seven plants were stripped of their slips and each lot constituted a separately marked family. All were fumigated with hydrocyanic acid, as described by Hagan (10). These slips were then set in culture solutions under cages designed to exclude all but the experimental insects (Fig. 6, A). The slips from 1 family were planted in sterilized soil under somewhat similar cages (Fig. 6, C). Forty-four slips from the 7 parents were used: 20 were checks of which 3 developed small mealybug populations, due to inadequate fumigation; 2 were infested with 1 gravid female, each; 7 with 10 mealy bugs of various ages and 15 with 50 mealy bugs, all of which came from a badly wilted pineapple field. The infestation time was varied, being made on 14 plants in September, a month after planting, and on 10 in November, 1930. The results of this experiment are presented in summarized form in table 1. The notes under "Appearance of plants" cover the whole period of the experiment, which lasted for approximately 10 months.

TABLE 1.—*Pseudococcus brevipes as a single factor in the production of pineapple wilt*

No. of plants	No. of insects applied to each plant	Appearance of plants	Green spotting	
			On old leaves	On new leaves
2	1	Normal	++	++
4	10	"	++++	+++—[a]
2	10	No typical symptoms	++	++
1	10	Partial wilt	+	+
9	50	Normal	On all	On all
1	50	No typical symptoms		
4	50	Partial wilt	+++	+++
13	None	Normal	On all	On 3[b]
6	None	No typical symptoms	++++	----

[a] Mealy bugs failed to establish.

[b] Small populations of mealy bugs developed.

Discussion of Experiment I. From the above table it is seen that new growth became green-spotted on all but the 1 plant on which populations failed to develop. Typical wilt symptoms developed in 2½ months on 1 plant and in a less pronounced manner on 5 others in from 4 to 6 months. The most typical case of wilt obtained in this experiment (Fig. 7, A) was removed from its cage and dissected. The green spots on the leaves, evi-

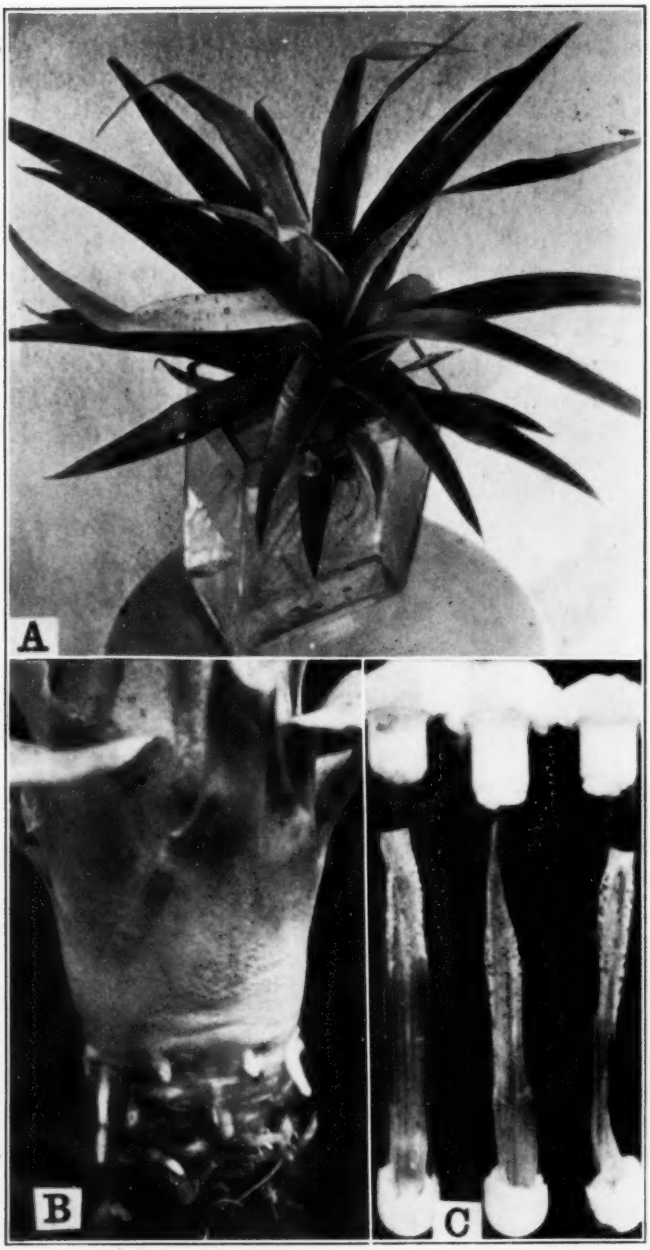

FIG. 7. A. Plant grown in cage (Fig. 6, A), showing typical wilt symptoms after infestation with mealy bugs. B. Plant of figure 4, B, 1 week after being trimmed and set in culture solution. Note new roots. C. Leaves from wilted plant used for tests on the effect of host sequence on toxicity of *Pseudococcus brevipes*.

dence of mealy-bug feeding, were counted for each whorl of leaves. These data are listed below, the whorls being numbered, beginning at the inside growing point of the plant.

Whorl 1. Only tips visible and they were necrotic. *Whorl 2.* First and 2nd leaves pink and flabby; 3rd leaf still green, except for browned tip: all covered with green spots. *Whorl 3.* All pink and flabby: covered with green spots. *Whorl 4.* First leaf—54 spots on new growth, which was green. 2nd leaf 169 spots: leaf pink at tip and flabby. 3rd leaf pink and flabby, covered with spots. *Whorl 5.* Two, 20 and 38 spots on new growth of leaves, normal except for slight paling. *Whorls 6 to 10.* Perfectly green and normal: scattered green spots on old growth; none on new.

From this case it appeared that a relationship existed between area actually fed upon and amount of tissue collapsed. Five other plants showing localized wilt indicated further the possible quantitative relationship between area actually fed upon and leaf collapse, the area of leaf collapsed being coincident with that heavily spotted. Some of these cases simulated a type of wilt frequently found in the field and designated as "terminal" wilt. In this type the ends of the median whorls of leaves are pink, often with sharp demarcation between normal and affected tissue, while the center whorls are normal. This indicated that such types were the result of a short-lived mealy-bug colony's feeding, which damaged tissue locally without any appearance of systemic effect. Some plants of this experiment showed green spotting on all the leaves that developed after infestation, for a period of 6 and 8 months, unaccompanied by any wilt symptoms. This indicated that green spotting *per se* was not a concomitant of wilt; neither did green spotting on planting material forecast later wilt of the plant. It will be noted that on only one of the 9 plants infested with less than 50 mealy bugs did typical symptoms develop and then only in a mild way, even though all these plants showed typical green spotting.

This last question was tested separately by growing a series of plants that had been so heavily infested as to leave the foliage covered with green spots. The material was fumigated to remove mealy bugs and grown in isolation cages. New growth was spot-free and perfectly normal in color, vigor, and growth. As was true in some of the plants of Experiment I, the old heavily spotted basal leaves yellowed and dried down.

Effect of absence of ants. It usually has been considered that the protective influence of ants was the major contribution to the mutual relationship of ants to mealy bugs. Experiment I, however, indicated clearly that a mealy-bug colony unattended by ants was abnormal. Masses of honeydew and cast skins accumulate and the distribution of the young is seriously impeded. Instead of remaining in the heart of the plant where the most succulent tissue is, these small masses of gummy mealy bugs remain

on a very small area of leaf as the leaf grows out, sometimes persisting even when the leaf has become shrivelled and brown (Fig. 6, B). It is significant in this connection that the most typical case of wilt obtained was one in which the mealy bugs promptly established themselves in the heart tissue.

This experiment established for the first time the rôle of *Pseudococcus brevipes* as a single and primary factor in the production of typical pineapple wilt. The low incidence was attributed to abnormal mealy-bug colonies, but the influence of the field complex was also considered, and an experiment was set out in the field plots. Progress in mealy-bug control gave some assurance that check plots could be kept clean by spraying (3).

Experiment II. Effect of adding mealy bugs to pineapple plants, growing under natural field conditions

This experiment considered 3 possible variables: size of initial infestation, time of infestation, and type of planting material. There were 2 cases in each class, as shown in table 2.

TABLE 2.—*Arrangement of Experiment II*

Planted	Infestation dates			
9/25/30	2/3/31		4/27/31	
Good planting material	1 gravid female per plant	50 mealy bugs, mixed sizes and ages	1 gravid female per plant	50 mealy bugs, mixed sizes and ages
Poor planting material	1 gravid female per plant	50 mealy bugs, mixed sizes and ages	1 gravid female per plant	50 mealy bugs, mixed sizes and ages

The selection of good and poor planting material was necessarily empirical, but the growth of the 2 types showed the expected differences in uniformity and vigor. There were 50 plots in all, containing 1,000 plants, of which 400 were scattered in 20 check plots. Each plot consisted of a section of a regular 2-line bed and contained 20 plants. This set-up was duplicated in another field, but the 2 areas showed essentially similar results. The better planting-material plots showed uniform growth; the poorer planting material was uneven, some being very slow in starting. These differences became less pronounced as the season progressed. The mealy bugs used in these tests were obtained from wilting plants and came from an area where green spotting was general.

There was rapid establishment of the colonies with noticeable differences obtaining, however, between the establishment on the plots planted with good planting material and some of the plants in the plots of poor planting

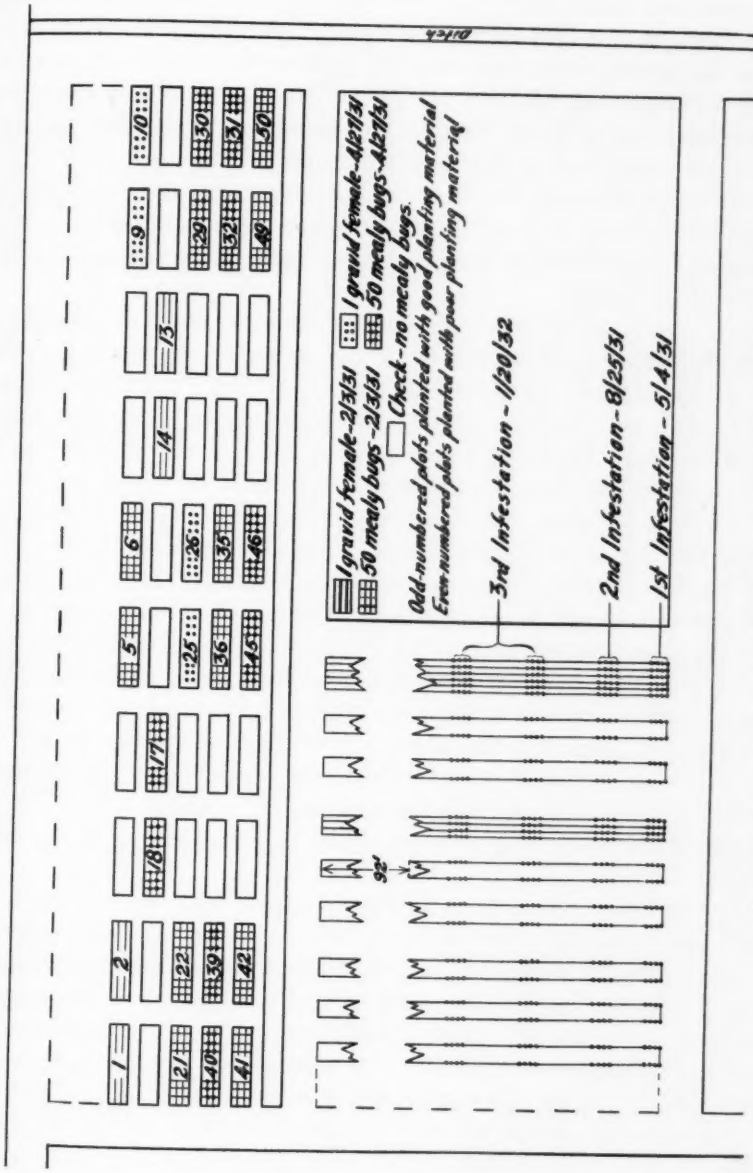
1 gravid female-2/3/31
50 mealy bugs -2/3/31
Check - no mealy bugs.
Odd-numbered plots planted with good planting material
Even-numbered plots planted with poor planting material
3rd Infestation - 1/20/32
2nd Infestation - 8/25/31
1st Infestation - 5/4/31
92'

FIG. 8. Arrangement of field plots in Experiments II and III.

material. The former, being uniformly succulent and vigorous, showed uniformly rapid establishment of the mealy-bug colonies; the latter included some individuals that were retarded, and these had tougher tissue. Establishment on these plants was noticeably less rapid. In the area included in the experimental plots, Pheidole ants were present and promptly attended the mealy-bug colonies put on the plants. In figure 8 is shown the arrangement of plots for this and a subsequent experiment, and the results of the experiment are presented in tables 3 to 5.

The results of this experiment, besides demonstrating the importance of *Pseudococcus brevipes* in producing wilt under field conditions, revealed something of the intricacy of the problem and gave some suggestions as to the nature of the injury.

The response of the plants to the infestation of 50 mealy bugs made on February 3, 1931, when the plants were 4 months old, was quite clear. Typical flabby collapse was evident April 27, 1931, on a considerable number and by July 6, 1931, the whole series showed almost complete collapse (Fig. 3, A and B). The mealy-bug populations were at this time extremely small, evidence of the fact that the bugs will desert a wilting plant.

The response to single mealy-bug-infestation experiments made on the same date was, however, quite different. These plants had, in the course of time, developed large populations of mealy bugs; yet the only evidence of plant response, except in the case of 5 out of the 80 plants in these plots, was in a slight spindliness of growth. These populations continued to increase until the basal portions of the inner leaves were literally covered by the colony. There was some variation in size of these colonies between 2 widely separated pairs of plots, which was of some significance in the end result. All these single individual infestations, however, developed populations far in excess of those responsible for nearly complete collapse in the case of plants that were subject to an initial population of 50 mealy bugs on the same day.

On April 27 the second series of plots was infested. Plots that, up to that time, had been kept clean of mealy bugs by means of sprays were infested with 1 gravid female per plant or 50 mealy bugs per plant, as in the first series. As before, Pheidole ants promptly appeared in attendance. The plants by that time, of course, were larger and more succulent than at the time the first series was infested. All were normal in color and growth. The results of this second infestation added more evidence as to the ability of *Pseudococcus brevipes* to produce a wilted condition of the pineapple plant, for, on July 6, approximately 10 weeks after infestation, some of the heavily infested plots showed complete collapse and all showed a high percentage of wilted plants (Fig. 3, C). The plants infested with 1 female, apart from developing considerable populations of mealy bugs during that time, showed no typical symptoms, except in 1 case.

TABLE 3.—*Wilt occurring after 1 gravid female mealy bug had been applied to each plant. Twenty plants in each plot. One series of plots infested February 3, 1931, and another on April 27, 1931*

Plots infested Feb. 3	Total number of plants wilted in each plot on following dates					Condition of plants as observed April 11, 1932			
	4/27/31	7/6/31	9/18/31	11/11/31	2/9/32	No recovery	Wilted in fruit stage	New leaves normal in color but spindly	Normal but small fruits
1 G	1	1	2	10	18	20	0	0	0
2 P	0	2	4	9	18	11	1	5	3
13 G	1	2	0	5	4	4	5	0	11
14 P	0	0	0	1	6	7	3	8	2
Plots infested April 27					2/3/32				
9 G	0	1[a]	2	—	3	9	0	0	11
10 P	0	1[a]	0	—	1	4	2	6	8
25 G	0	3[a]	—	10	9	4	5	3	8
26 P	0	2[a]	—	2	2	0	2	5	13

[a] With the exception of 1 of the plants of Plot 26, none of these plants showed typical symptoms but only reddening of the *outer* leaves.

TABLE 4.—*Wilt and recovery occurring after 50 mealy bugs had been applied to each plant. Twenty plants to each plot. Plots infested Feb. 3, when plants were 3 months old*

	April 27, 1931			July 6, 1931			Feb. 9, 1932			
Plot	Typical wilt	Plants retarded only	Plants normal	Plants apparently dead	Plants abnormal but still growing	Plants normal	No recovery	New growth normal in color but spindly	Recovery of terminal wilt type	Normal but retarded
5 G	16	3	1	17	2	1[a]	1[a]	19	0	0
6 P	7	5	8	12	8	0	0	14	0	6
21 G	20	0	0	14	6	0	1	19	0	0
22 P	10	0	10	12	6	2	3	15	0	2
1 G	3	0	17	17	2	1	0	11	9	0
2 P	2	0	18	14	6	0	0	14	0	6
9 G	19	0	1	20	0	0	0	7	13	0
10 P	10	0	10	14	0	6	3	13	0	4
15 G	1	0	19	14	3	3	0	3	10	7
16 P	0	0	20	5	14	1	1	0	4	15

[a] This plant is shown in figure 12 A and B.

TABLE 5.—*Wilt and recovery occurring after 50 mealy bugs had been applied to each plant. Twenty plants in each plot. Plots infested April 27 when plants were 5 months old*

Plot	April 27, 1931			July 6, 1931			Feb. 9, 1932		
	Typical wilt	Plants abnormal	Plants normal	Typical wilt	Plants abnormal but still growing	Plants normal	No recovery	New growth normal in color but spindly	Normal but retarded
175	1	6	13	14	6	0	0	20	0
18 P	0	0	20[a]	9	5	6	5	15	0
29 G	0	0	20	19	0	1	0	20	0
30 P	0	0	20	7	10	3	1	19	0
5 G	0	0	20	13	0	7	0	19	1
6 P	0	0	20[a]	16	0	4	3	10	7
11 G	0	1	19	19	0	1	0	20	0
12 P	0	0	20[a]	11	0	9	1	17	3
19 G	0	0	20[a]	16	0	4	2	18	0
20 P	0	0	20[a]	14	0	6	2	18	0

[a] Plants uneven in growth.

It will be observed that the plants infested with 1 gravid female showed little signs of collapse in any of the plots until November, 1931. In February, 1932, plants in plots 1 and 2 were nearly all wilted, while plots 13 and 14 still showed a low incidence. This difference was associated with great differences in populations. Counts made prior to collapse in plots 1 and 2 revealed that plots 1 and 2 were carrying populations of approximately 1,500 mealy bugs per plant, while populations on plots 13 and 14 were approximately 500 mealy bugs per plant. Observations made in April, 1932, showed plots 13 and 14 still superior to plots 1 and 2 in amount of recovery and numbers of normal plants.

Comparison of the plots planted to good and poor planting material and both heavily infested indicates what was obviously true on inspection, that the more vigorous succulent growth was more susceptible to wilt produced by a sudden attack of mealy bugs.

Plots 5 and 6 present additional data on this point. It will be observed that 8 plants were still growing on July 6, 1931. These plants had been noted as retarded and with poor establishment of the mealy bugs added 2 weeks after infestation. They continued to grow and, though somewhat smaller than the lightly infested plants, were, nevertheless, normal in color and growth 8 months later, by which time very large populations of mealy bugs had developed. In April, 1932, most of these plants were still normal in appearance except for retardation and evidence of heavy mealy-bug feeding on the leaves. The capacity of the plants to withstand a high population of mealy bugs over a long period of time, if the population develops from a low initial infestation, was strikingly illustrated in this experiment. On the other hand, the high toxicity of a large initial dose demonstrated beyond question the capacity of *Pseudococcus brevipes* to produce typical wilt of pineapple plants under field conditions.

In all these cases of wilt, in both light and heavy infestations, green spotting was pronounced and, in the case of the plants collapsing after a period of a year, following extremely heavy feeding, the leaves were covered with spots in various stages of development, from freshly made zonate spots to welts and areas of secondary necrosis.[3]

The behavior of the check plots was carefully observed, since, from them, suggestions as to other inimical factors could be obtained. There were 20 check plots in all, each containing 20 plants. In addition, up to the time of making the April 27, 1931, infestation, all those plots were also checks and at that time a few scattered plants showed symptoms of abnormality. This usually expressed itself as a reddening of the *outer* older leaves of the plant. Of the 400 plants in the checks on July 6, this redden-

[3] The descriptions of the types of green spotting and their development are too long to be included in this paper. They are considered more fully in another manuscript (5).

ing of the outer leaves was somewhat evident on 18, 10 of which were in one of the plots planted to the better grade of planting material. Samples of these were submitted to the Pathology Department for examination for pathogens, with negative results.

Six of the 400 were typically wilted, a condition that could not be ascribed to any known mealy-bug establishment, since these plots had been sprayed with oil emulsion at approximately monthly intervals. These 6 cases and others that followed were inexplicable until the data of the next described experiment were obtained, which indicated that these cases were the result of movement of mealy bugs from adjacent heavily infested plots. Observations made on August 18, 1931, revealed that all these cases were in direct apposition to wilted plots from which the mealy bugs had migrated.

The causality of the reddened outer-leaf condition remains unknown. As a factor, its low incidence and the fact that all the plants so affected grew normally after a short period of retardation suggest that its contribution to the collapse obtained in the infested plots was slight.

While the laboratory experiment (No. I) indicated the minor position of green spotting, with respect to wilt, the generally spotted condition of the wilted plants in this field experiment left the question a moot one as far as quick wilt was concerned. The next experiment recorded additional data on this question and, at the same time, revealed a previously unsuspected phase of the wilt problem.

On April 27, when the second series of plots in Experiment II was infested, an adjoining plot appeared to offer an opportunity to test the reaction of much larger plants under several conditions of planting. This plot had been laid out in 2-, 4- and 6-line beds as per figure 8. At the time of infestation, May 4, 1931, these plants were vigorous and normal in every respect. This plot was in the same field as Experiment II and planted at right angles to those plots, so that one end of each bed was contiguous.

Experiment III. Response of pineapple plants growing in various densities of planting to an initial infestation of 50 Pseudococcus brevipes

The inner ends were protected from infestation by virtue of their distance from infested plants, so the last 4 plants in each line of 9 beds were infested with 50 mealy bugs taken from wilting plants. At the time the 50 bugs were applied, these lines were free from ants. As frequently happens in the field, absence of ants means either poor establishment or dissolution of the mealy-bug colony. The infestations applied on May 4, 1931, did not establish and increase. The toxic dosage applied to these plants, therefore, was that imparted by the original 50 mealy bugs by feeding for a short time.

Observations made the latter part of June showed many of these plants "off color," that is, with the inner whorls of leaves distinctly reddened, and on July 6 a striking effect of the mealy-bug infestation was definitely evident. There was almost no evidence of movement of mealy bugs from the infested plants to the adjacent plants in the same lines except in 1 case where the 5th plant was infested and exhibited typical symptoms of a milder type (Fig. 2, A). The clean-cut division between infested and noninfested plants was so clear as to remove any remaining doubt as to the rôle of *Pseudococcus brevipes* in the production of wilt. Even more striking was the fact that the infested plants showed no evidence of mealy-bug feeding (*i.e.*, green spots) except in isolated cases, and, even in these, very careful examination was necessary to find them. In other words, a condition that had, in the past, been ascribed to a complex of root troubles because of the fact that no insect damage or insects could be found at the time symptoms were evident was here shown to be a phase of mealy-bug wilt. The symptoms of wilt in these cases were identical with those of Experiment II, except for the fact that green spotting was either extremely rare or absent. Table 6 summarizes the results of this experiment.

Relation of Age of Plant to Susceptibility to Quick Wilt. On August 25, 1931, the wilt in the foregoing experiment was still localized to the infested ends of each bed, the balance being vigorous and normal. A second section

TABLE 6.—*Response of pineapple plant growing in various densities of planting to an initial infestation of 50 Pseudococcus brevipes: Effect of age of plant on susceptibility.*

First section infested 5/4/31 Plants 6 months old		No. of plants collapsed		Notes on recovery			
Bed	No. of plants	7/6/31	10/2/31	February 16, 1932		April 11, 1932	
				Normal	Recovery	Normal	Recovery
2 line	8	8[a]	8[a]	0	5	0[b]	5
2 "	8	5	7	1	7	0	7 (1)[c]
2 "	8	4	5	3	5	3	5
2 "	8	6	8	1	7	1	6
2 "	8	4	5	3	5	2	6 (1)
4 "	16	12	13[a]	5	9	4	10 (1)
2 "	8	5	5[a]	2	5	2	5 (1)
2 "	8	5	7	2	5	1	6 (3)
6 "	24	14	20[a]	6	18	2	19 (6)

[a] Movement of bugs had occurred to 5th plant on one line. This plant, wilting.

[b] Fifth plant on one line now has small but normal fruit. All normal plants, this date, were fruiting.

[c] Figure in parentheses indicates number of recovered plants bearing small fruits.

TABLE 6.—(*Continued*)

Second section[a] infested 8/25/31 Plants 9½ months old		No. of plants collapsed			Notes on recovery	
					4/11/32	
Bed	No of plants	11/10/31	1/4/32	2/9/32	Wilted in early fruiting	Normal fruiting
2 line	8	2	4	4	3	4
2 "	8	3	2	3	3	5
2 "	8	3	7	6	5	1
2 "	8	6	5	5	3	4
2 "	8	3	2	2	1	7
4 "	16	10	10	12	11	5
2 "	8	8	8	8	8	0
2 "	8	4	7	7	7	1
6 "	24	9	12	14	11	12

[a] Third section infested 1/20/32—2 plots to each bed. Plants 14½ months old. 4/11/32 only one plant showing symptoms; it collapsed.

was then staked out in each of the beds (Fig. 8), consisting, as before, of 4 plants in each line. The plants at this time were 9½ months old. Mealy bugs from a wilting field were applied to these plants at the rate of 50 to each plant. At this time Pheidole ants were present and the mealy bugs established themselves.

The results of this second infestation are presented in table 7. There was considerable variation between beds in the amount of collapse ensuing and, on the whole, much less than occurred in the section infested May 4, when the plants were 3½ months younger. The reduced amount of collapse in the section infested August 25 was in spite of the fact that mealy-bug colonies established in that section and in April, 1932, were to be found in large numbers on the fruits. It was thus made evident again that the establishment of the colony was not necessary to ensure quick wilt, for in the first series practically complete collapse was obtained without establishment of colonies, while in the second series all the plants, not quick-wilting, developed large populations of mealy bugs.

The development of wilt in these last plots demonstrated that plants showing unmistakable symptoms of wilt prior to the appearance of the inflorescence could develop small inflorescences, which, however, wilted and dried up. This, a common occurrence in the field, was thus shown to be due to an abortive attempt at recovery by previously wilted plants. On the other hand, cases were obtained of this general type that represented the first appearance of wilt symptoms. In rare cases, recovery proceeded

to such an extent as to permit the development of a small but otherwise normal fruit.

Additional plots were marked out in the same beds (Fig. 8) and infested January 20, 1932, when the plants were 14½ months old. At this time the plants were in the stage just prior to the appearance of the inflorescence. The plots were duplicated in the same beds. Mealy bugs were taken from plants going into first ratoon[4] and showing symptoms of wilt. Establishment of colonies proceeded, but on April 11 only one plant was wilted.

Small colonies were to be found on the fruits at this time, but very much smaller than might have been expected from the size of the initial infestation and the length of time elapsing. The fact that colonies established themselves immediately after infestation, however, indicates that the plants were adequately exposed. Since only 1 case out of the 192 showed symptoms, it is evident that susceptibility to quick wilt decreases very rapidly with age of plant.

Quick Wilt not the Result of Local Injury. Results of Experiment III could not be explained on the basis of localized injury and suggested an extensive diffusion of an extremely toxic material. Proof of this was obtained when typical quick wilt resulted from the caging of mealy bugs on localized areas of leaves, the insects being placed as far down on the leaves as was possible without mechanical injury to the plant.

Comparison of the Toxicity of Mealy Bugs taken from Various Sources. The host-plant sequence of an insect is, no doubt, of importance in conditioning the effect of the insect on its host plant. This question is of general significance, for most, if not all, insect-transmitted or induced diseases of plants involve the movement or migration of the insects concerned from an alternate host or through a succession of hosts to the plant attacked.

It is true that with virous diseases the case is complicated by the reaction of the virus *per se* to the changed host conditions as well as that of the insect vector. Attenuation of the virus of curly top (1, 14) is probably primarily an instance of the former. A suggestion as to the possibility of the latter was made by Carter (2, p. 82). In the case of insects whose secretions are toxic as, for example, the leaf hoppers causing tip burn of plants, the probability of host sequence affecting the nature of the secretion of the insect is much greater and its demonstration involves less difficulty.

Experiment IV. Toxicity of mealy bugs as taken from the roots of 2 species of grass and from pineapple plants of various ages and conditions

In Experiment IV, mealy bugs were taken from 6 different sources as listed below:

[4] The suckers arising from the mother plant after the first fruit has been harvested form the plants of the first ratoon; those arising from the first ratoon plants, the second ratoon, and so on.

1. Red-top Natal grass, *Tricholaena rosea* Nees, growing along the edge of a pineapple field that had become infested from that source.
2. Healthy pineapple plants, growing a few feet away from the red-top grass. Green spotting was either rare or entirely absent from these plants. In the general area involved in both this and the following sample, high populations and wilt without green spotting was the general rule. The mealy-bug colonies here clearly represented edge infestation, as extensive sampling revealed its localization to that area of the field immediately contiguous to the grassy edge. It was difficult to find sufficient plants for this class, since remoteness from wilting plants was a desirable factor to include, and it was necessary to consider this factor in terms of a few feet.
3. Pineapple plants, showing initial symptoms of wilt. These were scattered along the edge of the field, rarely more than a few feet from the edge. It should be carefully borne in mind that the above 3 types had, according to all evidence, a common source of mealy bugs in the red-top Natal grass.
4. Panicum grass, *Panicum barbinode*, growing as an intercycle crop on a previously badly wilted field.
5. Young suckers of a coming first-ratoon pineapple field. These were taken from an area that had been under observation for some time and had contained high populations of mealy bugs the preceding year. There had been considerable collapse among the mother plants in this area and the suckers from which mealy bugs were obtained that were taken off for this experiment were all showing initial symptoms of wilt. Green spotting also was marked.
6. Small fruits and fruit tops, growing in an abandoned first ratoon field 1 year older than class 5. Tops were green-spotted. This was a typical field, abandoned because of wilt; most of the plants were wilted and the few remaining were characterized by tough tissue and had small stunted fruits. Considering the colonies obtained, from the standpoint of their nutritional history, there was probably considerable variation among them.

Using the above described insect material, 2 widely separated localities were chosen and plots arranged as per figure 9. One series replicated each treatment 3 times; the other, 6. Fifty mealy bugs were applied in September, 1931, to each plant in each plot, which included 20 plants, making a total of 1,080 plants in the 54 plots.

The results of this experiment are listed in tables 7 and 8.

Though the infestation conditions were exactly the same in these 2 locations, as far as initial infestations were concerned, the progress of the colonies differed materially. Location 1 was situated within a field a consider-

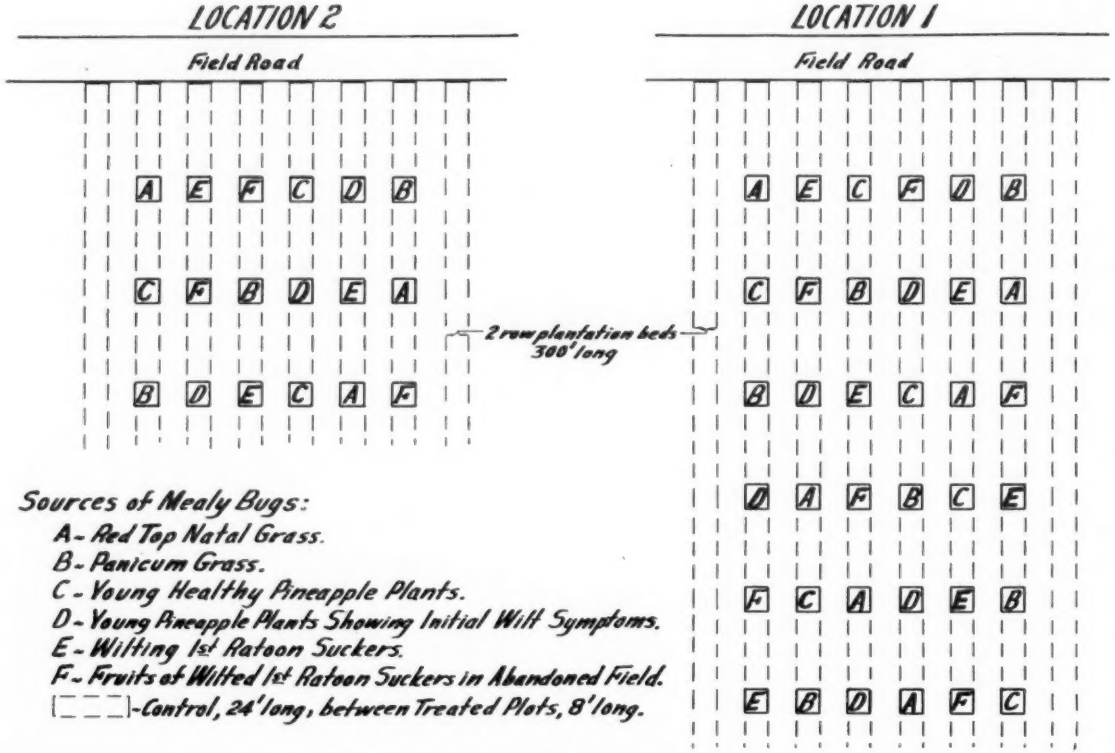
LOCATION 2
Field Road
LOCATION 1
Field Road
A E F C D B
C F B D E A
B D E C A F
2 row plantation beds 300' long
A E C F D B
C F B D E A
B D E C A F
D A F B C E
F C A D E B
E B D A F C
Sources of Mealy Bugs:
A - Red Top Natal Grass.
B - Panicum Grass.
C - Young Healthy Pineapple Plants.
D - Young Pineapple Plants Showing Initial Wilt Symptoms.
E - Wilting 1st Ratoon Suckers.
F - Fruits of Wilted 1st Ratoon Suckers in Abandoned Field.
[ ] - Control, 24' long, between Treated Plots, 8' long.

FIG. 9. Arrangement of plots in Experiment IV.

TABLE 7.—*Toxicity of mealy bugs from various sources. Plants infested during September, 1931. Total number of plants wilted in each series, at each date of observation*

Source	Location 1			Location 2		
	11/25/31	1/4/32	2/3/32	11/25/31	1/4/32	2/3/32
Red-top grass	0	6	6	2	3	5
Healthy pineapple plants contiguous to grass	2	4	11	2	6	11
Wilting pineapple plants from same area	3	53	66	6	30	41
Panicum grass	1	5	5	2	2	4
Wilting suckers from field going into first ratoon	24	73	80	21	51	53
First-ratoon fruits and tops from abandoned field	10	51	68	10	16	25

able distance from the edge. There were no ants in the vicinity. In addition to this, large numbers of *Conocephalus saltator* (Sauss.), a grasshopper reported (12) as occasionally predacious on mealy bugs, were seen in the plot. Examination of these plots the day after infestation showed establishment to be proceeding normally, but, prior to the first wilt count, careful examination revealed not a single colony established. In the other plots Pheidole ants appeared promptly after the mealy bugs were introduced. The 2 areas can thus be compared with respect to the influence of establishment of colonies on development of quick wilt. Location 1, containing twice as many plots as location 2, which was ant-attended, should have shown twice as much collapse if the establishment of colonies was entirely immaterial, a condition that did not hold true except in 1 case. The probability of extremely short-lived colonies in location 1 was great, and the results indicate that even quick wilt has a minimum dosage requirement.

From figure 10 it is seen that there was a gradual rise in toxicity, according to the source of mealy bugs. This toxicity reaches its peak in mealy bugs from young suckers from plant-crop mother plants. The reduced toxicity of mealy bugs from the ratoon crop, which was 1 year older, is possibly of some significance. If relative toxicity can be expressed in terms of time ensuing before collapse occurs, the mealy bugs from young

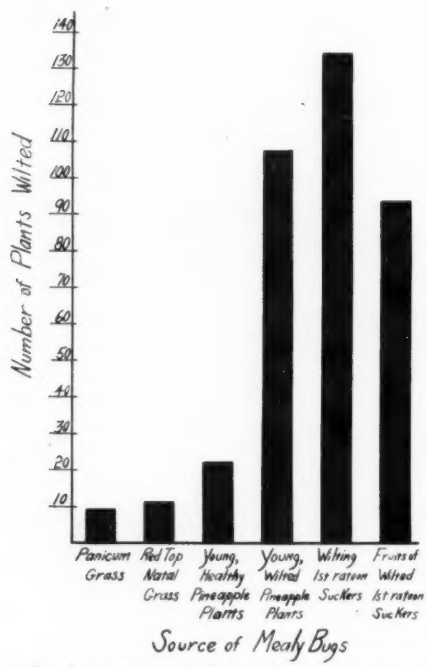

FIG. 10. Plants wilted when mealy bugs from various sources were used.

suckers were the most toxic in this respect also, since, on January 4, nearly all the collapse to occur took place in these plots.

Variability in the plots infested from any one source is of interest. This is shown in table 8, where individual plot totals are shown.

TABLE 8.—*Variation between plots infested with mealy bugs from the same source. Number of plants wilted in each plot 2/3/32, 5 months after infestation*

Source	Location 1						Location 2			Total of 9 plots
Panicum grass	0	0	1	1	1	2	0	1	3	9
Red-top grass	0	0	0	1	1	4	0	1	4	11
Healthy, young pine-apple plants	0	0	1	2	2	6	2	3	6	22
Wilting, young pine-apple plants	4	6	10	13	14	19	10	11	20	107
Wilting suckers	7	9	12	15	17	20	16	17	20	133
First-ratoon fruits and tops	6	7	10	12	13	20	6	8	11	93

These variations may be due to differences in susceptibility of individual plants. They may, however, be due rather to variations in the original mealy-bug infestations. The healthy plants used may have been previously infested for varying lengths of time and more or less close to showing wilt symptoms. The same holds with respect to the young plants in early wilt, since these differed considerably in the extent of evident wilting. The mealy bugs from young, wilting suckers were probably a more uniform sample than any of the others as to length of time spent by the colony on pineapples and also as to the stage of wilt developed in the suckers.

The material from ratoon fruits and tops was most variable; fruits were of various sizes and conditions, most of them stunted and growing on plants that varied considerably in succulence. The plots of this series varied most in amount of collapse. Examination of all these plots revealed no relationship between green spotting and amount of collapse. Mealy bugs from wilting young plants produced green spotting in only 1 plot out of 9 on January 4, while plots infested with mealy bugs from old, green-spotted ratoon-field plants showed green spotting to be general.

Preliminary tests of the effect of a reversed host sequence are described in Experiment V.

Experiment V. Preliminary tests of the effect of host sequence on mealy-bug toxicity

Since experiments on toxicity of mealy bugs from various sources had indicated a relationship between toxicity and host plant or host-plant condition, the following experiment was performed in the laboratory.

A mealy-bug colony from wilting suckers was divided into 3 parts. One aliquot was placed on Panicum grass and, after feeding thereon for 9 days, the mealy bugs were transferred to healthy pineapple plants, 50 to each of 5 plants. A second aliquot was transferred to the leaves of another pineapple plant in advanced wilt. These leaves were separated from the plant and kept in tubes, as shown in figure 7, C. After feeding on these wilted and drying leaves for 19 days the mealy bugs were transferred, 50 to each of 4 healthy pineapple plants. The third aliquot was transferred directly to healthy plants, 50 mealy bugs to each of 5 plants. The results of this experiment are shown in table 9.

A colony of mealy bugs from red-top Natal grass was divided. Some were placed directly on healthy pineapple plants, 50 to each of 5 plants. Another aliquot was fed on leaves from the same badly wilted and green-spotted pineapple plant used in the last-named experiment. After 11 days on these leaves, the bugs were transferred in the same manner to 3 healthy pineapple plants. Neither transfer resulted in either wilting or green spotting.

TABLE 9.—*Effect of host-plant sequence in modifying toxicity of mealy bugs*

Host sequence	Number of plants used	Wilt ensuing
Wilting suckers direct to healthy plants	5	4[a]
From wilting suckers; to Panicum grass for 9 days; thence to healthy plants	5	0[a]
From wilting suckers to leaves of a plant in advanced stages of wilt of 19 days; thence to healthy plants	4	1 partial wilt[a] Edges of leaves on remaining plants reflexed inward

[a] All these plants were green-spotted.

In view of the small number of plants used, the evidence of these experiments can be considered only as preliminary. It is, however, rather clearly indicative. The wilt obtained was typical and pronounced, so the complete absence of wilt in the plants fed on by the mealy bugs from wilting suckers *via* Panicum grass indicates the profound effect of changes in the insect's nutrition on the toxicity of its secretions. The fact that mealy bugs will forsake a wilting plant is evidence of the unsuitability of such plants as hosts; so, the results obtained by forcing the insect to feed on a wilted leaf for a considerable time are similarly indicative of a modification of the insect's secretion.

Comparison of Green-spotting and Nongreen-spotting Mealy Bugs in Relative Capacity to Produce Wilt. Field observation had shown that, while green spotting was generally to be found in wilted areas, it was not necessarily always associated with wilt, even under field conditions. As has been mentioned, some of the mealy bugs used in the above experiment came from wilted plants showing no green spots. Other areas on the island of Oahu revealed the same to be true, even though of rare occurrence. On the island of Kauai, however, there are areas that have been under intermittent observation for the last 2 years and have shown no green spotting, although typical wilt associated with high mealy-bug populations is common. A laboratory experiment was, therefore, set up, using mealy bugs from wilting plants from 1 of these areas on Kauai in comparison with typical green-spotting mealy bugs from wilting plants on Oahu.

The conclusion was definitely established from this experiment that typical wilt of pineapples follows feeding of *Pseudococcus brevipes* irrespective of green spotting. The development of the wilt obtained in both series indicates clearly the limited extent of the injury, since, with the great

reduction in size of colonies, most of the plants had normal center leaves after 5 months.

The results indicate, however, that in the presence of green spotting, wilting is somewhat more severe; though such evidence as there is on this point may be explained as due to the much more rapid establishment of the green-spotting colonies. The green-spotting colonies established more rapidly and have maintained themselves with much greater vigor than the non-green-spotting colonies.

In May, 1932, these latter colonies were reduced in every case to a few scattered individuals, while the green-spotting colonies were vigorous and, in some cases, very large. Evidence of the existence of biological strains of mealy bugs is considered in more detail in another paper (5). The matter is mentioned here, since such strains, or perhaps even physiological states, of a colony, may be of importance in determining the toxicity of the colony to pineapple plants.

Recovery of Wilted Plants. This is a phenomenon that has been consistently encountered during the course of this study. Notes are included in tables 3 to 7 on the recovery of plants under several conditions of infestation and subsequent collapse. In all the infestations of 50 mealy bugs to the plant made in Experiment II on February 3, 1931, recovery followed the apparently complete collapse of the plant. This manifested itself in the production of new leaves in the center, normal in color, but very narrow and somewhat brittle, giving the effect of spindly growth noted in the tables. This new growth, while of no significance commercially, is of great interest in that it demonstrates that the meristematic tissue was not injured.

The plots infested April 27, 1931, with 50 mealy bugs showed this recovery in a much greater degree. The most clean-cut example is shown in figures 4, A, and 11. This series of photographs of the same plot illustrates the gradual recovery from an apparently complete collapse. A laboratory experiment was performed on 5 of the plants from this plot, which accounts for the blanks seen in figure 11. These plants were pulled and the dead roots and old butts trimmed off. The plants were then set in culture solutions. All promptly produced apparently normal roots. This series is shown in figures 4, B, and 7, B.

The recovered plants in this plot, at the time of photographing, figure 11, B, harbored colonies of mealy bugs that obviously totaled more than the 50 of the original infestation. It should be recalled that the colonies developing on these plants practically disappeared with the collapse of the plants, so that the colonies found on the recovered plants represented a gradual building up from the few remaining on the plants. The fact that the plant could recover to the extent shown is a remarkable instance and is strongly suggestive of an antitoxic or accommodatory reaction on the part

FIG. 11. A. Same plot as in figure 4, A. Photographed Nov. 20, 1931. B. The same plot photographed Feb. 9, 1932.

of the plant. On April 11, 1932, 1 of the plants in this plot was beginning to wilt for the second time; the balance showed no wilt symptoms.

Recovery on the plots of Experiment II was of interest in indicating the relationship between age of the plant at the time of collapse, and sub-

sequent recovery. Observations, made April 11, showed that recovery had taken place on the plants of the first infested plot to the extent of permitting the development of small but otherwise normal fruits on 3 plants and apparently normal but small inflorescences on 13. The plot infested 3½ months later showed 6 plants wilting on February 16, which, on April 11,

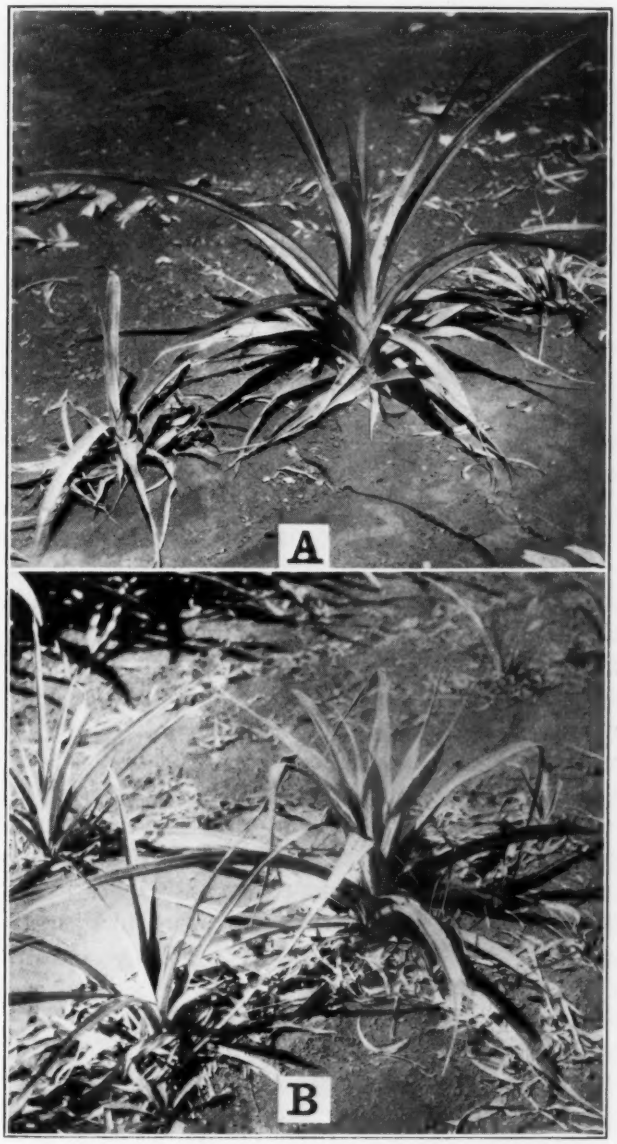

FIG. 12. A. Plant showing resistance to quick wilt. July 4, 1931. B. Collapse of plant in A, from slow wilt Nov. 20, 1931. Note recovery of contiguous plants.

were recorded as bearing normal fruits. All the other cases of wilt recorded February 16 in this plot showed either no further growth or an attempt at recovery, which culminated in a small inflorescence, at which point the plant ceased growing and the inflorescence dried up.

When pineapple plants have collapsed from slow wilt, recovery apparently does not occur. This was particularly evident in plot 1 of table 3. The other plots of this series showed some spindly recovery, but these were the plants that had been the first to collapse. Plot 5 of table 4 had 1 plant that did not develop quick wilt, while all the surrounding plants did. On July 4, 1931, this one plant was outstanding, as shown in figure 12, A. Following this, a very large colony developed, although the plant attained large size. In the meantime the collapsed plants in the same plot began to show spindly recovery. On November 20, 1931, as shown in figure 12, B, the large plant had collapsed from slow wilt.

Three other expressions of recovery should be mentioned. If the mealybug colony is short-lived or disappears entirely after collapse of the plant, the center whorls of leaves are normal in color, except for reddened or chlorotic areas at the tips. As the plant grows the new leaves are normal and the originally affected whorls show tip reddening only. This type is frequently encountered within fields where colonies have started on infested planting material and have died shortly afterwards. Another type follows recovery from quick wilt in 8- to 10-month-old plants (Figs. 1 and 5, A, and B). It is characterized by brittle, narrow, new, central leaves with a reddish brown cast. This condition is more likely to be confused with plant failures due to other causes than any of the previous types. The third type is characterized by the occurrence of wilt in early fruiting; the production of normal suckers in these cases is a commonly encountered phenomenon. It should be borne in mind that none of these recovery types are of any significance commercially.

Evidence for the Plant's Accommodation to Mealy-bug Feeding. As has been shown in Experiment II, tables 3 to 6, a suddenly applied colony of 50 mealy bugs produced wilt quickly in plants from 6 to 11 months old. On the other hand, some plants infested with 1 gravid female grew to a large size, even though colonies comprising over 1,500 individual mealy bugs developed before the plant finally succumbed to wilt. Some plants, similarly infested, but that had by November 20, 1931, developed colonies of approximately 500, produced a large percentage of normal-size fruits. Since the mealy bugs were all taken from the same source, these differences cannot be explained on a basis of difference in individual mealy-bug toxicity when the insects were first placed on the plants.

To test this, some of the mealy bugs from one of the large colonies were taken on December 17, 1931, from a still healthy plant in plot 1 and each

of the plants growing in soil and under cages was infested with 50 of these mealy bugs. Another lot of plants was similarly infested with mealy bugs from the most toxic source known, *i.e.*, wilting first-ratoon suckers. There were 9 plants in each lot. Observations made up to April 19, 4 months after infestation, revealed essentially the same results from each. Complete as well as partial collapse occurred in both series in the same lapse of time. It was evident from this experiment that both lots of mealy bugs were equally toxic.

Evidence of the ability of the plant to withstand a large population of mealy bugs for a long time, if the colony started from a very small initial infestation, can be commonly seen in any of the pineapple areas in Hawaii. The fact is of great importance, for, while quick wilt is extremely difficult to stop, once mealy-bug colonies are established on pineapple plants, the holding down of mealy-bug populations to a very low level is practical and is being accomplished. Further experiments on the subject of the accommodation of the plant to mealy-bug attack are now under way.

The Possible Nature of the Injury. Mealy-bug wilt of pineapple appears to present a type of insect-induced plant disease for which it is difficult to find a parallel case, although there are points of similarity with hopperburn of potatoes (7) and sugar-cane froghopper blight in Trinidad (18, 19). There is no positive evidence of either a virus or a "transmissible" disease, transmissible in the sense of being transferred by insect "pick up" from 1 plant to another. On the other hand, there is definitely negative evidence to the contrary.

Vegetative planting material from areas that have collapsed shortly after removal of the slips has been used for years in the ordinary plantation practice. This planting material has almost invariably been infested, at time of planting, with mealy bugs from the same colonies as those that produced wilt in the parent plants. The relationship between size of initial infestation and later behavior of the plant indicates clearly that a time-quantity relationship holds. The remarkable recovery of the plant is further evidence that we are not dealing with either a virus or a transmissible disease, in the limited sense. It would seem that the injury is more likely to be a toxic secretion that is diffusible but incapable of reproducing in the plant. The accommodation of the plant to large but gradually developed colonies is inexplicable if a living systematically distributed entity is postulated as causal.

The speed and completeness of the collapse in the case of plants that have been subject to a sudden and heavy attack indicate a systemic condition, but the recovery of these plants as shown in figures 4, A, and 7, B, shows that, even in those cases, the toxic principle did not kill either the

root primordia or the meristematic tissue at the base of the new leaves. The cases of terminal wilt appear to be examples of restricted diffusion of the toxin.

Whether quick and slow wilts are expressions of the same causality cannot be definitely determined now; the latter case may be due to a combination of a weakening of the plant's accommodation or the gradual elimination of the functional leaf tissue or both. The latter, as a single factor, is unlikely in view of the fact that the plant can withstand the removal of a large proportion of its leaf tissue without untoward effects.

Data on the influence of source of mealy bugs and subsequent plant collapse indicate differences in degree rather than kind and suggest what seems to the writer to be a reasonable hypothesis of great significance if true. That is to say, the toxicity of the insect's secretion varies with the nutritional conditions under which the insect is living. This toxicity decreases after reaching a peak attained shortly after plant-crop harvest, so that mealy bugs from old ratoons are actually less toxic than those that have developed on the extremely succulent plant-crop tissue. No conclusion as to the nature of the injury is yet possible. The question has merely been opened up for further work.

Mealy Bugs in Relation to Other Inimical Factors. Although *Pseudococcus brevipes* has been shown by laboratory experiments to be a primary and single factor in causing wilt, with symptoms similar to those found under field conditions, it still remains true that, in the latter case, mealy bugs are operating in the presence of a biological complex that may condition their effect on the plants. Since time-quantity relationships hold for mealy-bug toxicity, it follows that the presence of other inimical factors might reduce both the amount of mealy-bug infestation necessary and the time over which it operates to produce wilt. On the other hand, mealy-bug-wilt symptoms might mask the effects of other factors that might, themselves, be important contributors.

Evidence is accumulating, however, from a large series of control experiments, as well as from commercial fields that have been consistently treated for mealy-bug control, that mealy-bug wilt is the major factor in failure of pineapples in Hawaii and that other factors only operate in extremely localized areas and sometimes then only under abnormal weather conditions.

SUMMARY AND CONCLUSION

The pineapple mealy bug, *Pseudococcus brevipes*, has been shown by laboratory and field experiments to be primarily responsible for a widespread collapse of pineapple plants. Since symptoms are characteristically of the wilt type, the term "mealy-bug wilt" is a suitable designation.

Green spotting of pineapple leaves is a localized effect at the insect's feeding point and not a typical wilt symptom. Wilt is more pronounced, however, where green spotting is present. Ants are important contributors to the vigor of a mealy-bug colony and the incidence of wilt is low where these species, notably *Pheidole megacephala* and *Solenopsis geminata* var. *rufa*, are absent.

The disease assumes many forms, dependent upon the size, time of onset of the initial mealy-bug population, the vigor and succulence of the plant, and the fact that recovery in various degrees is commonly encountered.

Quick wilt is the type that follows a sudden attack by a number of mealy bugs. Approximately 2 months are required for development of typical symptoms. The establishment of the mealy-bug colony is not necessary to produce quick wilt, a short period, only, of feeding by the insects being sufficient. Under these circumstances there is a rapid diffusion of the toxic principle. This diffusion may be localized to the leaf area actually fed upon or it may affect 2 or 3 leaves or the entire plant. It does not, however, kill the meristematic tissue either of root primordia or younger leaves. Susceptibility to quick wilt decreases under conditions of reduced vigor and succulence and age of the plant.

Recovery from quick wilt is commonly encountered. It is usually of no commercial significance but sometimes results in the production of small but otherwise normal fruits.

Evidence of antitoxic reactions on the part of the plant is available, for when a colony develops from a single gravid female, no quick wilt occurs, even though enormous numbers of mealy bugs are feeding on the plant. If such a colony continues over a period of many months, slow wilt may occur, from which there is no recovery. The size of the colony and the length of time it operates determine whether slow wilt will occur.

There is definite evidence that the mealy bug's toxicity varies with the kind and condition of the insect's host plant. This is believed due to changes in the insect's secretions induced by the varied nutritional conditions afforded by the host plants.

Mealy-bug wilt appears to be an insect-transmitted disease for which no closely parallel case is known. The evidence points to a nonliving toxic insect secretion of variable diffusibility as causal.

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THE SPOTTING OF PINEAPPLE LEAVES CAUSED BY PSEUDOCOCCUS BREVIPES, THE PINEAPPLE MEALY BUG[1]

WALTER CARTER

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INTRODUCTION

The green spotting of pineapple plants caused by the feeding of pineapple mealy bugs, *Pseudococcus brevipes* (Ckl.), is conspicuous in many pineapple areas in Hawaii. It was considered a characteristic symptom of pineapple wilt by Illingworth (3). Experimental evidence obtained by the writer and recorded in another manuscript (1) led to the conclusion that "typical wilt of pineapple follows feeding of *P. brevipes,* irrespective of green spotting," even though wilt appears to be more severe in areas where green spotting is the rule. This conclusion is also supported by much field observation. For the purpose of this study, therefore, the phenomenon of green spotting has been considered as something separate and distinct from pineapple wilt. For purposes of comparison, the injury following the feeding of nongreen-spotting mealy bugs was studied also.

TYPES OF SPOTTING RESULTING FROM MEALY-BUG FEEDING

A. *Green-spotting Bugs.* Like other members of the genus, *Pseudococcus brevipes,* though capable of active locomotion, normally feeds at one place on the leaf for some considerable time. If a leaf is pulled out of the center of an infested pineapple plant, the mealy bugs can be seen *en masse* near the white tissue at the proximal end of the leaf. This relative position is maintained unless the colony becomes very large, in which case it extends up the leaf for some distance.

In this white tissue, and especially at the poorly defined junction of green and white tissue, faint yellow-green homogeneous spots appear. As the leaf grows out, these spots retain their relatively darker color than the surrounding tissue. By the time the leaf area in which they are has become normally green these spots are clearly defined, but they vary a good deal both in initial appearance and development. Early laboratory tests indicated that green spotting was localized around the insect's feeding point. Tests to determine this were made by caging mealy bugs on small areas of pineapple leaves, as shown in figure 1, A. Spotting consistently occurred, but only on those areas where the insects had fed.

[1] Published with the approval of the Director as Technical Paper No. 49 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

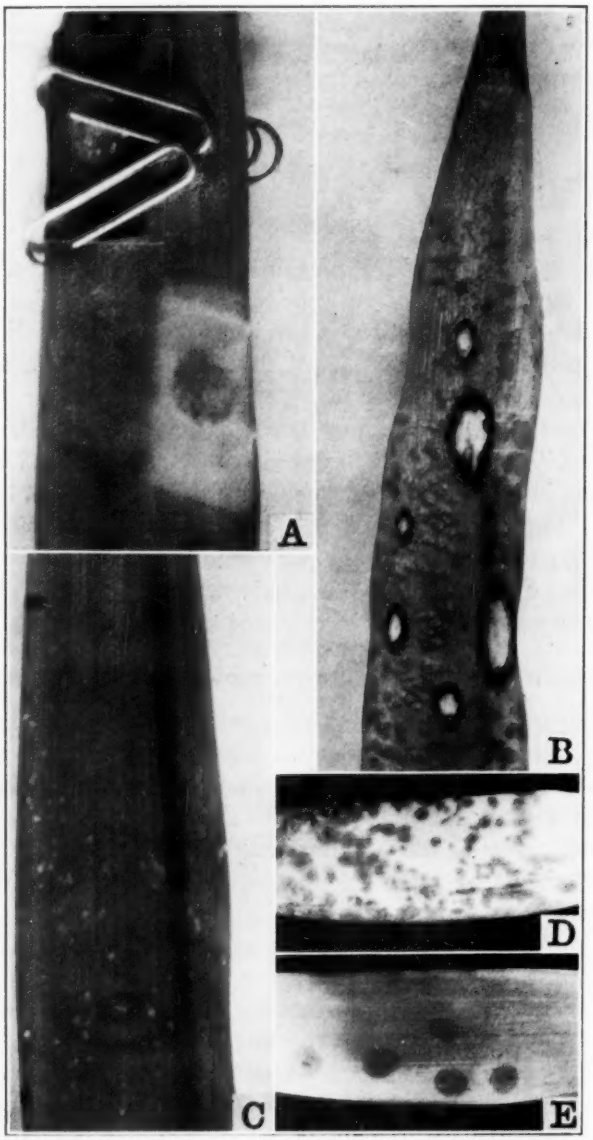

FIG. 1. Portions of pineapple leaves, A and D being from seedling leaves. A. Area with a felt-celluloid cage for confining mealy bugs and one showing green spots confined to portion that bugs fed upon. B. Homogeneous spots on margins, welt-like spots in median area and areas of secondary necrosis. C. Disappearance of outer green zones from leaf marked when spots were normally zonate. D. Fixation of color in mass of green spots and extremely chlorotic areas between them. E. Large green spots with dark centers. At left note the spot in the chlorotic tissue at proximal end of leaf.

The principal forms taken by these spots are listed below and illustrated in figures 1, B–E, and 2, D.

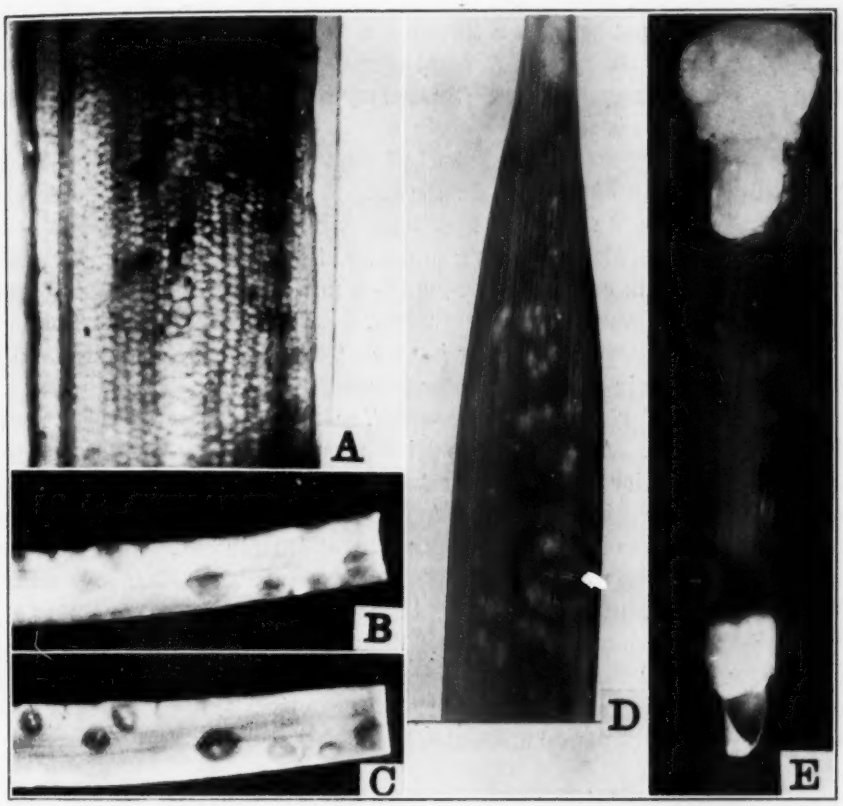

FIG. 2. A. Section through chlorotic spot, showing thickened cell walls and densely staining areas filled with homogeneous mucilage-like substance. B. Green spots with dark centers on seedling leaf. C. Same leaf as in B, after clearing in 95 per cent alcohol and staining in iodine-potassium-iodide solution. Note dense staining of green spots and their light unstained centers. D. Grouping of chlorotic spots in areas of irregular margin. E. Tubed leaf of pineapple seedling used in laboratory experiments.

1. A faint, usually circular, homogeneous spot of yellow-green in the white tissue of the leaf. Frequently 2 or more in a small group. *2.* Homogeneous, usually circular, darker green area in the young, green tissue; sometimes slightly depressed. *3.* As in 2, but with a concentric zone of lighter green around the darker center. This lighter zone is still darker green than the normal tissue. Frequently the outer zones of 2 or more of these spots will merge. The zonate character of a spot may appear with the first sign of spotting, or much later. *4.* Green welts. The green spot

becomes slightly raised in somewhat older tissue and is particularly conspicuous in chlorotic leaves that have shrivelled. Occasionally these welts develop a small browned center of secondary necrosis. 5. The darker center of the zonate spot becomes chlorotic and the outer zone either disappears entirely or nearly so. When the leaf shrivels and becomes chlorotic these spots show as faint green welts. Spots may be chlorotic-centered from the beginning, but this is rare.

The spots vary from approximately 1 mm. to 10 mm., the average being from 3 to 5 mm. in diameter. In the largest one encountered, the darker center was ovoid and measured 4×2 mm. Size of spots is sometimes associated with a particular mealy bug or group of mealy bugs. One small colony produced spots on a seedling leaf 6–8 mm. across. This size of spot was maintained through several transfers, finally, however, appearing normal in size. When a large colony is present the leaf is sometimes literally covered with spots in all stages of development.

B. *Feeding Marks of Nongreen-spotting Bugs.* These are of a much simpler character. A nongreen-spotting mealy bug leaves either no definite mark or a chlorotic spot, sometimes circular but usually of irregular margin and very variable in size. A large colony will sometimes cover the leaf with such areas so that it appears almost translucent. In these cases there is usually some rugosity due to the irregular shrinking of the leaf. The chlorotic spots usually develop a brown necrotic center.

While an individual green spot is the result of the feeding of a single mealy bug, the chlorotic area is more usually the result of a mass of mealy bugs feeding in one very localized area for some time—several days, or even weeks, in the case of young and succulent plants.

PRELIMINARY STUDIES OF THE ANATOMY OF THE SPOTS

In view of the variety of forms of green spotting and the complexity of the problem as revealed by transmission studies, anatomical studies have been limited to the grosser features with a view to comparing the chlorotic with the green-spotted area as a means of establishing the specific identity of the 2 types.

Mealy-bug Feeding Tracks. The feeding tracks left by Homopterous insects in plant tissue have been studied in detail by other workers (2, 4, 6). The tracks of *Pseudococcus brevipes* are readily found in both types of spotting. They are remarkably uniform in width and surface. They penetrate intracellularly to the region of the fibrovascular bundles where they may branch, each branch leading to a separate bundle. Rarely a track may pass through the entire width of the leaf, curving back without interruption to a point near the fibrovascular bundles where branching may occur.

No differences in general morphology have been observable between feeding tracks in green and chlorotic-spotted areas. In view of the conclusion reached that the 2 types are the result of differences in secretion, a comparative microchemical study of feeding tracks should prove fruitful.

The Chlorotic Area. Sections through these areas show degenerated chloroplasts (Figs. 2, A, and 3). When stained with iodine-potassium

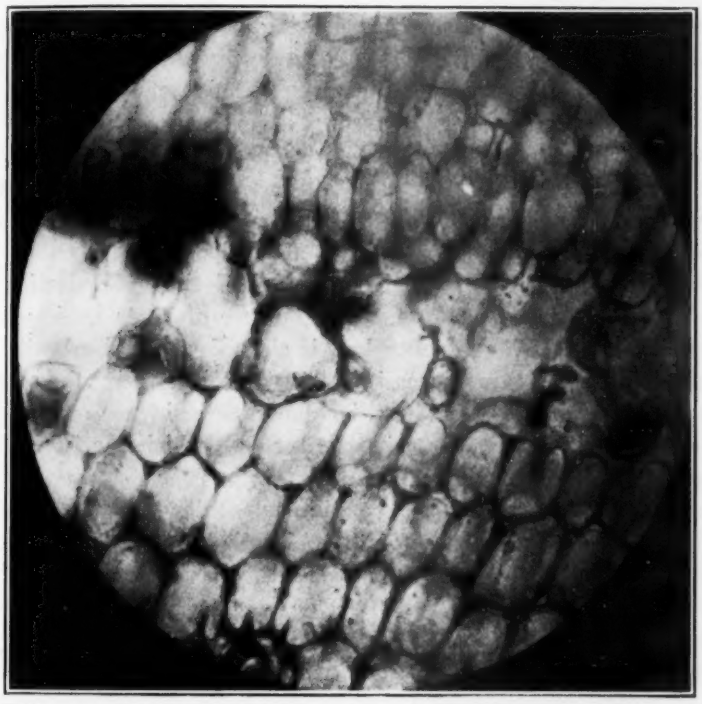

FIG. 3. **Enlargement of portion of figure 2, A, showing degenerated chloroplasts, cell-wall thickening, and densely staining cell contents.**

iodide it is seen that the area is free of starch. Stained with dilute safranin, a thickening of the cell walls can be clearly seen. This varies in extent, being more pronounced in the central portion of the area and especially so when the center of the spot appears browned with secondary necrosis. This central area, in section, showed heavier vein thickening from the epidermis to the fibrovascular bundles. The finding of old feeding tracks in this area suggests the probability of its developing around the feeding track, the reduced thickening towards the periphery being due to a diffusion of the secretion in that direction. Within this area of pronounced vein thickening, many cells are full of a homogeneous nongranular substance that takes a slightly less dense stain than the thickened cell walls. Differences be-

tween chlorotic areas on the same leaf and on different plants appear as slight variations in the extent of vein thickening.

Green Spots. These present an entirely different morphological picture. In the earliest stages, when the green spot shows as a faint yellow-green area in the white tissue, sections show the area to be a zone of hyperactivity. The chloroplasts are clearly formed within the area that is surrounded by normal white tissue in which no chloroplasts are to be found. Stained with iodine-potassium iodide, after clearing in 95 per cent alcohol, the chloroplasts in the area of the spot become deeply stained, so that the contrast between it and the surrounding tissue is heightened.

Green spots within normally green leaf areas show this same deeper staining with iodine-potassium iodide with a diffused muddy-brown color throughout the area of the spot, as well as the deep-stained chloroplasts. When stained with 1 per cent acid fuchsin without clearing the leaf, a diffused pink fills the area. When stained after clearing, it is pale lavender, with scattered cells throughout the normal leaf tissue filled with diffuse pink. There is a curious difference in the case of spots having a deeper green central zone. When such a leaf is cleared and stained with iodine-potassium iodide, the stained area is exactly coincident with the area of the spot except for the central zone, which shows no evidence of starch (Fig. 2, B and C). This central area is that through which the insect's mouth parts actually punctured the leaf.

No evidence has been obtained to indicate thickening of cell walls or other injuries, as seen typically in the chlorotic area, in any of the forms of green spot. The typical condition in these is enlargement and increase in number of chloroplasts. When tissue is cut from a green spot and macerated on a slide, flat plate-like polygonal bodies, with irregularly thickened edges, are to be found. The bodies stain deeply with iodine-potassium iodide. Similar treatments of normal tissue from the vicinity of the spot show these to be absent.

The green welt, isolated in a completely chlorotic area at the drying tip of a leaf, presents a curious picture. In such a spot the cells appear still to be living, even though the cells of the surrounding tissue are dead and shrunken.

The Occurrence of the Two Types in the Field. Field observations on the islands of Oahu and Kauai suggested that green spotting was not necessarily a concomitant of mealy-bug feeding, even where pineapple wilt was present. In a considerable portion of the pineapple area on Kauai, no green spots could be found, even in badly wilting plants. In other places green spotting is rare, only very small pinhead-size spots being found and they only occasionally on any one plant. In other areas, notably in the drier lands, green spotting is the rule. Plants carrying nongreen spotting

colonies are occasionally found on all the islands of the group, but green spotting in greater or less degree is the rule on all the islands but Kauai. Observations on an experimental plot on Oahu in the spring of 1931 revealed large colonies present but no green spotting along a field edge of approximately ½ mile, except in 1 small plot. Typical wilt was associated with the chlorotic type of feeding spot. Occasionally, 2 plants growing in extremely close proximity will show green spots on the one and not on the other. Green spotting will sometimes appear on the plants for the first time on the crowns of the fruit.

Mealy bugs from hosts other than pineapple were tested for green spotting. Two of these host plants, Panicum grass, *Panicum barbinode*, and red-top Natal grass, *Tricholaena rosea* Nees, are important sources of mealy-bug infestation since they are frequently found on the edges of fields, and colonies from these 2 species have been maintained on pineapple seedlings over a considerable period. No positive evidence of green spotting by colonies from either grass has yet been obtained. Two species of sisal are typically green-spotted by *Pseudococcus brevipes*, and transfers of the insects to pineapple resulted in the prompt green spotting of the leaves. *Pseudococcus brevipes* from the roots of purslane, *Portulaca oleraceus* Linn., and Rhodes grass, *Chloris gayana* Kunth, produced green spots when transferred to pineapple-seedling leaves. The negative evidence obtained from a number of other hosts must be considered as only tentative in view of the later finding that the capacity to produce green spots may be limited to certain individuals in a colony.

As these differences in the field became evident, samples of the mealy bugs found both on pineapples and other hosts were brought to the laboratory for study.

Technique of Laboratory Studies. Seedling-pineapple plants were used, grown in soil, and enclosed in glass jars with cloth tops. Mealy bugs from the field were transferred to these and the development of spotting noted. In order to study the comparative effect of individuals of the same colony, some of these seedlings were dissected and the largest leaves placed in a small quantity of culture solution in a sterilized test-tube (Fig. 2, E). This type of tube proved most useful since green spots developed readily and reproduction of the mealy bugs took place. Sterilizing of the prepared tube prior to enclosing the leaf very much reduced secondary infections, but the type of culture solution was immaterial. With the rapid corking of the proximal end of the leaf it is doubtful whether the solution does more than prevent desiccation.

The seedlings were grown in the glasshouse while the tube colonies were held under various laboratory conditions of light and temperature. Green spots developed on leaves placed in total darkness at the time of infestation

and also on leaves held in total darkness for 1 to 2 weeks prior to adding the insects. Most of the experimental colonies grown in tubes were placed upright in cans under conditions permitting diffused light only to reach the leaves.

EXPERIMENTAL WORK

The first question was whether green spotting was merely a reaction of individual plants. This was tested in 2 ways: first, by feeding green-spotting mealy bugs on the leaves from which nongreen-spotting mealy bugs were removed; second, when mealy bugs failed to produce green spots on seedlings, the seedlings were dissected and the individual leaves tubed. Some of these leaves were then reinfested with the same bugs as a check on the changed conditions under which the leaf was then growing, while green-spotting bugs were transferred to the balance of the separated seedling leaves. These experiments resulted consistently in evidence that the difference between green and nongreen spotting was due to differences between mealy bugs.

Establishment of a Consistently Nongreen-spotting Colony of Pseudococcus brevipes. A mealy-bug colony taken from a first-ratoon sucker growing in a Haleakala Ranch field on the Island of Maui had produced no green spots on the field plant. The colony was transferred to seedlings and later to a long series of tube colonies. The pedigree of this colony is illustrated in figure 4. It definitely establishes the fact that *Pseudococcus*

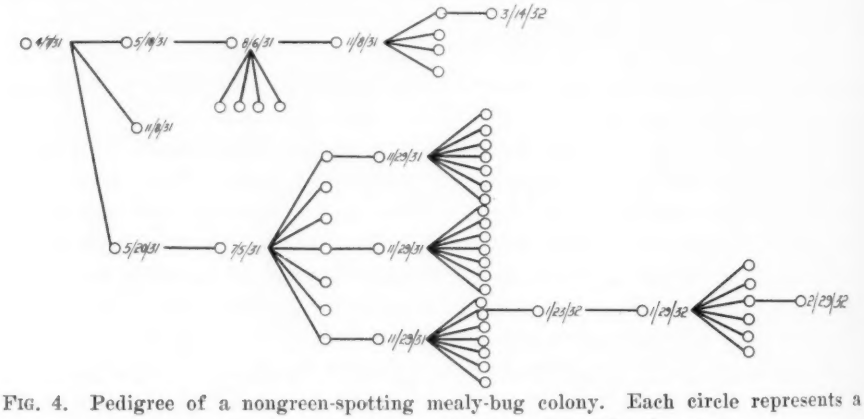

FIG. 4. Pedigree of a nongreen-spotting mealy-bug colony. Each circle represents a separate colony grown on seedlings and seedling leaves.

brevipes colonies may exist for a long time and on a long succession of pineapple seedlings without any green spots resulting.

Another pedigree of a nongreen-spotting colony from the field edge on Oahu mentioned above presents some interesting possibilities. It will be seen (Fig. 5, B) that occasional cases of green spotting appeared. This

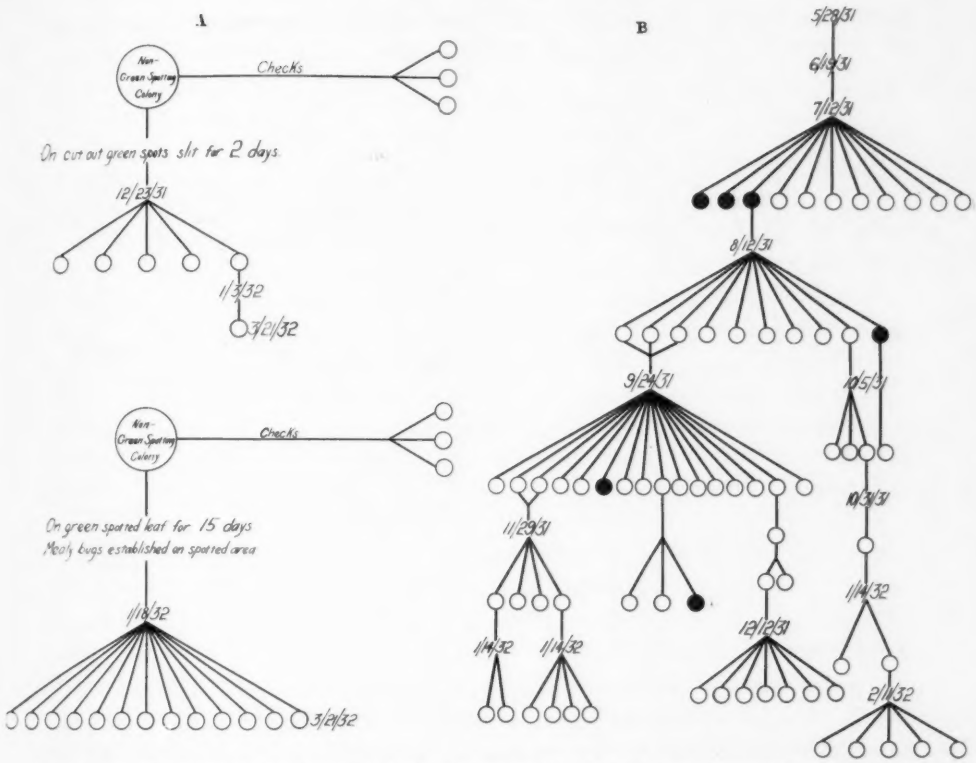

FIG. 5. A. Experiments on transmission of green spot. Each circle represents a separate colony on seedling leaves, none of which showed any green spotting. B. Pedigree of a mealy-bug colony, showing occasional occurrence of green spotting. Green-spotted plants are shown as solid circles.

indicates the possibility of colonies being inherently capable of producing green spots but having this capacity in a recessive condition.

Continuity of Green Spotting by Colonies and Individual Mealy Bugs. Colonies have been maintained on single plants for periods of up to 1 year with a continued successive appearance of green spots. Occasional examples have occurred where green spots have failed to appear on the new growth, but, when the insects were transferred to new plants, green spots appeared on them some time later. Colonies removed from wilted pineapple plants that were badly green-spotted on all but the newest tissues gave variable results. Some aliquots of the original colonies produced green spots on young seedlings and others not. It should be noted in all these cases that the colony rather than the individual mealy bug has been the unit.

Individual mealy bugs have been fed on a succession of separated pineapple leaves, being transferred after feeding for various lengths of time. This series is shown in table 1.

TABLE 1.—*Sequence and number of green spots obtained from individual mealy bugs*

Series	Days of feeding							
	1	3	6	3	9	6	3	8
1	0	0	0	1	2	0	0	
2	1	0		0	0	Progeny produced spots	Progeny produced spots	
3	3	1	3	1	2	0		
4	1	1	1	5	7	4	1	2
5	0	1	1	0				
6	1	1	1	1	1	2	1	Progeny produced spots
7	0	0	0	2				
8	1	1	1	0	0	2	0	
9	1	1	1	2	1	2	0	0
10	1	1	0	1	0	Progeny produced spots		Progeny produced spots
11	1	0	2	0	0	0	0	0
12	0	0	0	0	0	0	0	0

In this table the number of green spots developing on each leaf is shown. It will be observed that there was a good deal of variation between individual insects, 1 individual not producing a single spot during the whole period. This is of interest, since all these mealy bugs came from a very profusely spotted leaf. They were, however, variable in size and age and several became mature during the course of the experiment. It is apparent in following the course of mealy-bug development that the gravid female feeds but little prior to the emergence of her young, so that negative results towards the end of the insect's development may be primarily due to reduced feeding.

The green spotting by the progenies of 3 individuals should be noted, since it occurred on the same leaves on which parent insects failed to produce spots.

Development of the Green Spots. Since green spots are determined by comparison with the surrounding tissue, the initial density of the chlorophyll and the rate at which the leaf becomes chlorotic are factors that affect the time elapsing between feeding of the insect and the appearance of the spot. When data of table 1 were obtained, records of the time elapsing between the first application of the insects and the development of the spot showed this to vary from 3 to 55 days. Occasionally, the spot develops while the insect is still feeding, but usually the spot appears after removal

of the insect. It seems true that a green-spotting bug moves its feeding point much more frequently than one unable to produce spots.

TRANSMISSION EXPERIMENTS

When it became evident that capacity to make green spots was limited to certain mealy bugs, experiments were performed on the transmissibility of green spotting.

Mechanical inoculation with macerated plant tissue, containing spots and with mealy bugs that had produced spots, was performed on growing seedlings and on separated seedling leaves. A large series gave negative results. In one case a large circular green spot resulted from hypodermic injection of macerated green-spotted tissue. This was interpreted as a wound reaction with which the metal of the needle might have been concerned, inasmuch as deep green areas frequently resulted from cracking of the leaf as it was being inserted into the tube and from the insertion of glass capillaries, which resulted in small necrotic areas with margins greener than surrounding tissue.

Experiments on transmission of green spot by first feeding nongreen-spotting individuals on green-spotted leaves gave negative results.

In the first of these experiments green spots were cut out of a leaf and partly slit with a razor blade. This was done to encourage the localization of the mealy bugs' feeding to the deeply spotted portion, since *Pseudococcus brevipes* has a tendency to establish in cracks and crevices. This localization occurred and the insects fed for 2 days before being transferred to seedling leaves (Fig. 5, A).

A second experiment using nongreen-spotting mealy bugs was then set up. Gravid females were placed on a heavily spotted leaf. The larvae from these females settled down on green spots and fed thereon for 15 days. They were then transferred to seedling leaves as shown in figure 5, A. Although the results of these transfers were all negative, they were of considerable significance in view of the success obtained in localizing the feeding of the insects to the actual green-spotted tissue.

THE HEREDITARY NATURE OF THE CAPACITY TO FORM GREEN SPOTS

This species of mealy bug, being viviparous, gives birth to living young. Gravid females, when placed in a glass container, such as a Petri dish, will give birth to numbers of these larvae, which can, therefore, be obtained without any previous feeding on plant tissue. Particular care was exercised that no larvae were adhering to the parent insects when the latter were transferred to the Petri dish. When unfed larvae from green-spotting mothers were transferred to clean seedling leaves extensive green spotting occurred. This experiment was repeated twice with the same results. Table 2 gives the data on one of these experiments.

TABLE 2.—*Production of green spots by larvae that had not previously fed*

Larvae transferred to seedling leaves 11/23/31	Observation dates		
	12/20/31	1/3/32	1/14/32
Seedling A.1[a]	−	+	
.2	+		
.3	+		
.4	−	−	+
.5	−	+	
.6	−	−	+
.7	−	+	
B.1	+		
.2	−	−	+
.3	−	+	
.4	+		
.5	+		
C.1	+		
.2	−	+	
.3	+		
D.2	−	+	
.4	+		
.5	+		
Larvae transferred to seedling leaves 11/26/31			
E.1	+		
.2	+		
.3	+		
.4	+		
B.6	+		

[a] Each decimal number represents a single separated leaf. The leaves of 5 seedlings were thus used in this experiment.

The dates when the larvae were removed are given, as well as the dates on which green spotting occurred. Removal of larvae from the glass dish on the 2 dates shown was for the purpose of testing the persistence of the green-spotting capacity through the entire brood. The results of the last removal show this to be the case.

EVIDENCE FOR EXISTENCE OF GREEN-SPOTTING STRAINS

In the colony pedigrees presented in figures 4 and 5, B, it is seen that colonies were maintained for a year without the appearance of any green

spots; in others, with occasional incidence of green spotting. Other colonies have consistently green-spotted the leaves, with some evidence of fluctuation in numbers of spots produced in any one period.

Observations in the field during January, 1931, revealed what was believed to be a high percentage of fungous parasitism of mealy bugs. Some large gravid females were black from the filling of the body with fungus spores. These were comparatively rare, but numerous other individuals could be differentiated from the rest of the colony by a distinct grayness as compared with the normal rose pink of the other individuals. These gray individuals were presumed to be in early stages of the disease, so samples were isolated on separated leaves in tubes. A similar set was made up, using typically pink individuals from the same large field colony. Six weeks later examination of both sets revealed that 4 out of 5 of the gray colonies had established and produced apparently normal young. In every case, green spots were present on the leaves. The pink colonies also had reproduced, but in not a single case were there any green spots.

Further field observations showed gray individuals present in all colonies found on green-spotted plants and only pink ones where no green spotting was evident. It is almost invariably true that the typical pink forms greatly outnumber the gray on any one plant. Colonies grown on a single plant for many months may show no green spotting on the bases of the new leaves, even though all the old leaves and the upper portions of the central whorl may be literally covered with green spots. This indicates fluctuation within the colony. On transfer of these mealy bugs to a new plant, green spots may appear, indicating either a change in the status of the individuals transferred, possibly stimulated by the fresh plant, or the development of the green-spotting capacity by the progeny.

Attention was then turned to approximately 160 colonies growing on seedlings in the greenhouse. These were from a great many sources; from pineapples from the various islands of Hawaii and from a number of wild hosts that were being tested as reservoirs of green spot. An examination showed that in all but 5 cases the association of the gray form with green-spotting colonies held true. In these 5 cases the colony was either absent or very small. No gray forms were encountered in any of the nongreen-spotting colonies.

Preliminary rearing tests show that the progenies of gray individuals are all normally pink at first and that some of these will produce green spots, while others will not. The gray forms do not assume that aspect until they are half-grown or even later.

The data indicate some interesting lines of investigation. The rearing of long series of progenies from both forms, with records of each individual

with respect to color and green spotting, is necessary to establish whether or not the gray form is a genetic expression or whether it is indicative of a physiological state of the colony or both.

The facts that it is possible to differentiate colonies by a macroscopic difference in body color and that these colony differences are associated with such profound variations in secretion of the insects as green spotting and nongreen spotting seem to indicate are of great significance in studies of the nature of the toxicity of insect secretions to plants.

DISCUSSION, WITH PRELIMINARY EVIDENCE, ON A HYPOTHESIS CONCERNING A POSSIBLE FUNCTION OF THE SYMBIONTS OF PSEUDOCOCCUS BREVIPES

There appear to be two separate and distinct localized effects of the feeding of *Pseudococcus brevipes* on pineapple leaves. One of these, the irregular chlorotic area, is the result of rather prolonged feeding, usually by a number of individuals feeding at one place. Its general character and gross anatomy suggest that it is the result of exhaustion of the cell contents. Its area does not appear to extend much beyond the actual area fed upon. It is closely allied to spotting caused by the feeding of other Coccidae, such as *Diaspis bromeliae* Kern. and *Pseudococcus longispinus* Targ., and probably represents the normal type of mealy-bug feeding injury. The green spot is clearly in quite a different category. It appears to have a definite developmental history, beginning as a zone of hyperactivity, continuing as an area in which the chloroplasts are larger and more numerous, and ending as a welt-like simulation of a gall. Its zonate character indicates diffusion, perhaps of specific secretion components, outward from the darker center of the spot. The fact that frequently this outer zone of paler green is lacking indicates the possibility of variations in the character of the secretion, even in green-spotting individuals. When one considers the colony pedigrees as shown in figures 4 and 5, B, it is evident that the capacity to produce green spots is clearly specific to certain colonies when these colonies are considered as units. When individual mealy bugs are tested, it is shown that not all the members of a green-spotting colony can produce green spots at any one time. Certain individuals, distinguishable on account of their distinctly brown body color, which produces a grayish cast to the waxy covering, are regularly able to produce green spots. Their progeny, at first normal pink in color, are not all able to produce green spots, though some have this capacity. It is not yet known whether or not all those producing green spots develop the atypical grayish cast. The evidence is clear, however, that there are great variations, even within the progeny of a single female.

If the chlorotic spot is the normal type of mealy-bug feeding injury it would seem that green spotting is a special case superimposed on the nor-

mal one. This is suggested by the iodine-staining reaction of green-spotted tissue (Fig. 2, C), and the disappearance of the intense green, leaving a chlorotic spot in place of the dark center (Fig. 1, C). It would seem also that green spots are formed only normally either at or very close to the white tissue at the proximal end of the leaf. When green-spotting mealy bugs are caged on the older tissue, chlorotic spots result.

The mealy bug, though possessed of functional legs throughout life, and ambulatory at will, is, nevertheless, a sedentary insect. If undisturbed it will, under normal conditions, remain at one feeding point for most of its life. A green-spotting mealy bug, on the other hand, appears unable to remain long in one place, a fact suggesting that the secretion rapidly renders the area unsuitable for further feeding. It is, nevertheless, true that green-spotting colonies appear to grow more vigorously on pineapple plants than do nongreen-spotting colonies, but no data are available on the relationships between this vigor and the presence of gray individuals.

While negative evidence obtained in mechanical inoculation cannot be considered as final, the evidence on insect transmission is conclusive and of very great interest. The failure of nongreen-spotting mealy bugs to become capable of forming green spots after long feeding on green-spotted tissue, coupled with the fact that larvae of a green-spotting parent are able to produce green spots on the first leaf on which they feed, is evidence that green spotting is not the result of infection of the plant by a specific organism but is, rather, a response by the plant to a secretion of the mealy bug, which is specific to certain individuals and not common to the species.

The secretion is, no doubt, the end result of the operation of some internal factor on the physiology of the insect. The hereditary nature of this factor, however, leads to a consideration of the insect's symbionts as being concerned. The presence of symbionts in more or less highly developed mycetomes or "symbiotic organs" in Coccidae has been known for many years and a rich literature on the subject is available. As far as the writer is aware, no suggestion as to the possible relationship between these organisms and the secretions of the host insect, particularly with reference to pathological effects on plants, has been made.[2] For that reason, the following preliminary evidence is presented, since it offers some support to the working hypothesis that the activities of the symbionts affect the insect's secretions.

[2] Rambousek's suggestion (5) that the mycetome plays a definite part in the spread of virus diseases is interesting but difficult to reconcile with the fact that the Homoptera as a group possess mycetomes with included symbionts, irrespective of whether they transmit virus diseases or not. Since that author's paper appeared, the transmission of virus diseases by thrips has been shown by several workers. This fact further negatives Rambousek's suggestion.

All the individuals of the species possess symbionts contained in organized mycetomes. With respect to green spotting, therefore, a fundamental premise must be that either the insect is disymbiotic or the symbionts belong to a single polymorphic species, this polymorphism being paralleled by variations in the physiological effects.

Comparison of the mycetomes of gray and pink mealy bugs, particularly when the pink forms are obtained from a colony in which no gray forms are present, reveals differences that can readily be seen with the naked eye. The mycetomes from definitely gray forms are brown, while those from pink forms present the normal appearance of freshly dissected white tissue. Under low-power magnification the brown mycetomes appear denser and with a more clearly defined membrane. They are less easily damaged in dissection.

Comparison of a long series indicates that the development of this type of mycetome is progressive and all stages can be found in a mixed colony of pink and gray forms, great variation being encountered in mycetomes from individuals determined by inspection as "pink."

Study of the included organisms reveals a common type and, in addition, a second form whose incidence varies according to the status of the colony with respect to green spotting. This second type of organism is bacterium-like in form. It is found intracellularly in the mycetocytes. Thus far, it has been possible to consistently demonstrate its presence in large numbers in the mycetocytes of gray forms; rarely, in pink forms in a mixed colony. On 2 occasions only, in colonies from pineapple plants that had a record of not producing green spots, were these organisms found. Other colonies of this type, as well as those direct from red-top Natal grass, have been consistently negative.

There are some curious parallelisms between green spotting and mealy-bug wilt that should be mentioned. It has been shown (1) that mealy bugs vary in their capacity to produce wilt according to the host plant from which they are taken, those from the roots of red-top Natal and panicum grasses being least toxic.

Direct transfer of mealy bugs from these 2 grasses has thus far failed to demonstrate green spotting. There are evidences of fluctuations in toxicity of mealy bugs according to the age and condition of the plants from which they are taken, while a progressive development of green spots both in size and type occurs. A degree of parallelism between these last two is thus indicated. When the plants accommodate a very large population of mealy bugs, green spots of type 5 are commonly encountered.

The progressive development of the mycetome and the incidence of the bacterium-like inclusions suggest an angle of approach to the question of the parallelism between green spotting and mealy-bug wilt.

CONCLUSIONS

Pseudococcus brevipes causes 2 general types of spotting on pineapple leaves. One is the chlorotic area commonly associated with Coccid feeding. The other is an entirely different type known as green spot. This assumes many forms. It has a distinct developmental history. Colonies of mealy bugs have been maintained for long periods with no green spotting occurring; for similar periods with the constant occurrence of green spots; or a colony may produce green spots at intervals.

Mealy bugs from nongreen-spotting colonies have failed to produce green spots after feeding on green-spotted tissue.

The ability to produce green spots is transmitted from the mother to her young and is limited to certain individuals in the colony. Not all the progeny of such individuals can produce green spots.

Preliminary evidence justifies the working hypothesis that the insect's secretions are conditioned by the activities of the mycetome and its included symbionts.

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THERMAL DEATH POINT OF HETERODERA RADICICOLA IN RELATION TO TIME[1]

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Recent investigations on the environmental relations of the root-knot nematode *Heterodera radicicola* (Greeff) Müller[2] at this station include studies on the relations of moisture, light, and temperature to this organism in its different stages. The studies of moisture and light relations are reported in another paper (13). The present paper covers in detail the investigations with controlled temperatures, given in part in a brief preliminary report (12).

There is reported in the literature very little exact research on this phase of the environmental relations of *Heterodera radicicola*. Stone and Smith (15) gave 60° C. as the soil temperature necessary to kill the nematode but stated that for practical purposes it would be advisable to use 82° to 100° C. because of the irregularity of heat penetration in large areas of soil. Bessey (2) concluded that heat was the most efficient means of eradicating nematodes from the soil. Garman (10) reported that dry heat and steam were satisfactory agents for disinfecting soil infested with carnation-root-knot nematodes. Cobb (6) recommended the use of steam. Frandsen (9) stated that at 40° C. every egg and larva was killed at the end of 18 hours. Beinhart (1) reported the need for maintaining a soil temperature of from 72° to 100° C. for ½ hour to sterilize seed beds. Melchers (14) stated that a temperature between 88° and 93½° C. should be maintained for at least ½ hour for controlling nematodes. Byars and Gilbert (5), in connection with hot-water sterilization, reported that, for effective killing of nematodes, a soil temperature of about 55° C. must be maintained for 30 to 60 minutes.

Duruz (7), investigating the effects of temperature on the activity of this nematode, reported greatest activity between 18½° and 29½° C. and that larvae and eggs were killed at 38½° C. Godfrey (11) reported results on the effects of temperature on the development of root knot. Tyler (16)

[1] Published with the approval of the Director as Technical Paper No. 38 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

[2] Since this paper has been in form for publication the following paper has been received: GOODEY, T. On the nomenclature of the root-gall nematodes. Jour. Helminth. 10: 21–28. 1932. Goodey presents with convincing evidence the invalidity of *radicicola* as a specific name for this organism. The writers prefer to wait, however, until possible questions as to the validity of the suggested new name are settled before adopting any change in nomenclature.

reported on the effects of temperature on the length of life history. There is no report of exact work on time and lethal-temperature relations.

METHODS

In the tests for determining the lethal temperatures for the larvae and eggs moisture and light conditions favorable to life were maintained. The apparatus used was a thermostat-controlled water bath with a turbine stirrer. A standardized thermometer graduated to 0.1° C. was kept at a constant level in the bath for temperature observations. Pyrex-glass test-tubes, 70 mm. long by 18 mm. in diameter, capacity about 2 cc., containing water up to 10 mm. from the brim, were suspended in the bath by test-tube holders. The temperature of the water in these tubes was checked as to equilibrium, with that in the bath, for each new adjustment. The larvae or eggs were inserted into the tubes in a small drop of water by means of a pipette. Temperatures were not reduced more than 0.1° C. by this process. Temperature fluctuations were never greater than 0.5°. At the end of each period of exposure the vials were removed and immediately placed in a bath of ice water in order to prevent further action of the heat on the organisms. The drop to room temperature took place in a few seconds. The possible effect of the sudden drop in temperature brought about by this method has been considered. The writers feel that this has not been a factor in the killing. Repeated tests were made in which larvae were changed from room temperature (22° C.) to actual freezing in a few seconds, and back to room temperature again, without damage to the nematodes. Other tests, in which lethal temperatures for very short (distinctly nonlethal) periods were tried, likewise showed no injurious effect.

Examinations of larvae were made within a few hours, after each exposure to controlled temperature, and results recorded. Further examinations were made at intervals to determine if apparent killing was only temporary injury. Eggs were removed and incubated according to standard procedure. Controls were kept under favorable moisture, temperature, and light conditions by placing them in a drop of tap water on a slide on a moist filter paper in a Petri dish, which was left in the laboratory. Readings on these were taken at the same time as those for exposed organisms.

The usual 10-minute exposure used in bacteriology was given in preliminary tests. In addition, larvae and egg masses were subjected to a graduated range of higher and lower temperatures, and the minimum period for killing was determined for each. One hundred newly hatched, active larvae were employed in every case in which larvae were tested; 2 egg masses were used in the case of the eggs. Each test with larvae was repeated 5 times and each one with egg masses 6 times. Results were very consistent. Averages only are reported.

EXPERIMENTAL

The results of the several tests on time and temperature relations are given in compact form in the accompanying tables. Instead of incorporating the figures for all replications, and for the sake of greater compactness, averages only are given without probable errors. The figures on percentage survival for periods shorter than the one that was 100 per cent lethal are remarkably uniform in many cases in the different replications. Furthermore, the averages show a logical trend toward lower percentage survival as the time increases. A more exact statistical analysis of the averages, which would be brought about by calculating probable errors, is not justified by the needs of the case. The intervals of time between readings naturally vary widely at different temperatures. The lower temperatures, at which complete killing required hours, and in the case of egg masses, days, were given much wider intervals than the higher temperatures, in which complete killing was attained in minutes or even in seconds. In the tables the second column is devoted to the time interval between readings, the subsequent column headings being the number of such intervals from the start of exposure. To compute the time necessary for complete killing, then, it is necessary to multiply the interval period by the number of such intervals that have elapsed.

The true period at which complete killing was attained is one-half the interval below the time at which complete killing was first recorded, plus or minus half the interval. Thus in table 1, at 42° C., for example, complete killing was attained at 22.5 minutes, plus or minus 2.5 minutes. Table 1 gives the results obtained from the hot-water treatment of *Heterodera radicicola* larvae, there being 5 replications, 100 larvae to a tube, for each temperature.

Heterodera radicicola larvae have complex protoplasmic organization and probably wide differences in thickness of the cuticle, size of body openings, etc. The result is considerable variation in resistance to heat. An attempt to determine whether or not there is a relatively straight-line relationship between percentage survival and time, when plotted on semilog paper, according to the method of Bigelow (3) with certain bacteria, later used by Esty and Williams (8), is shown in figure 1. At the intermediate temperatures and the intermediate time intervals, it is evident that such relationship exists to some extent. Within limits, such a graph can be used to determine the length of time required to kill this nematode at any temperature within the range of lethal temperatures.

The eggs, as they occur in egg masses, show a much higher resistance to heat than do the larvae. This is shown to advantage in table 2, which is prepared in the same manner as table 1, there being 6 replications of each

TABLE 1.—*Percentage of survival of Heterodera radicicola larvae*

TABLE 1.—*Percentage of survival of Heterodera radicicola larvae at different temperatures and different periods of time (average of 5 readings)*

Temperature °C.	Interval between observations	Number of interval after exposure											
		1	2	3	4	5	6	7	8	9	10	11	12
40	15 min.			100.0	100.0	78.8	38.6	23.4	15.2	0.0	0.0		
41	10 "	99.4	14.4	1.6	0.2	0.0	0.0						
42	5 "	100.0	36.2	24.4	1.6	0.0	0.0						
43	1 "			100.0	100.0	99.8	98.6	87.8	0.0	0.0			
44	15 sec.										100.0	100.0	97.8
45	15 "						100.0	100.0	99.4	98.6	98.2	84.2	62.2
46	15 "					100.0	100.0	99.0	94.2	80.4	53.4	0.2	0.0
47	15 "												
48	5 "	100.0	100.0	99.4	99.8	98.4	96.8	95.8	94.8	84.0	56.8	17.8	0.0
49	5 "	100.0	100.0	99.0	98.4	97.4	96.0	94.8	92.0	70.8	51.2	13.2	0.0
50	5 "	100.0	100.0	98.8	93.6	89.8	88.6	85.0	7.6	6.0	3.4	0.0	0.0
51	1 "					14.0	5.0	0.0					
52	1 "	24.0	0.0										
53	1 "	0.0											

Temperature, °C.	Interval between observations	Number of interval after exposure												
		13	14	15	16	17	18	19	20	21	22	23	24	25
40	15 min.													
41	10 "													
42	5 "													
43	1 "													
44	15 sec.							100.0	100.0	98.4	79.4	20.0	0.0	0.0
45	15 "	92.8	88.2	82.2	61.4	41.2	32.4	20.4	0.0					
46	15 "	22.2	5.6	0.2	0.0	0.0								
47	15 "	0.0												
48	5 "	0.0												
49	5 "	0.0												
50	5 "													
51	1 "													
52	1 "													
53	1 "													

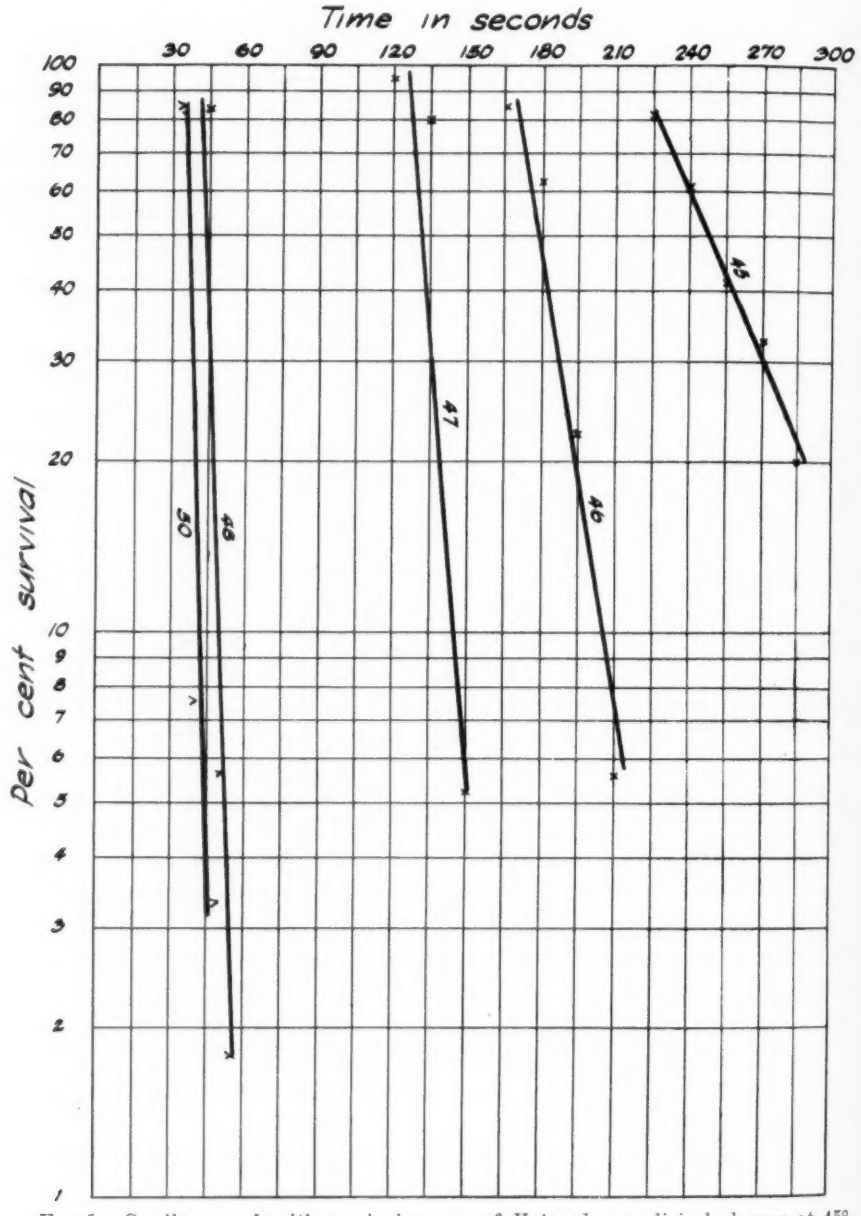

FIG. 1. Semilog graph with survival curves of *Heterodera radicicola* larvae at 45°, 46°, 47°, 48°, and 50° C. While the high ends of the curves are somewhat irregular, the intermediate points show a striking straight-line relationship.

temperature treatment, 2 egg masses in each tube. The averages of the 6 repetitions only, without probable error, are here recorded as being sufficiently accurate for all needs. For heat treatments designed for complete killing of nematodes the 0.0 per cent survival temperatures are, of course, the significant ones, such temperatures being required for complete efficiency in killing. At 40° C., the lowest lethal temperature for both larvae and eggs, 4.5 days (± 0.5 day) were required to kill all the eggs, as compared with only 127.5 minutes (± 7.5 minutes) in the case of larvae. Other temperatures are in proportion. At 50° C., complete killing occurs at 3.5 minutes, ± 0.5 minute. There was instantaneous killing at 58° C., with killing at 57° in 3 seconds and at 56° in 5 seconds. These last figures are only approximate because of the human equation, but they are clearly sufficiently accurate for all practical purposes.

No attempt is here made to show the log-graph relationship between time and percentage of egg survival. It is certain from a few trials that irregularities were considerably greater than with larvae.

The data on "thermal death times" of eggs in egg masses are plotted according to the method of Bigelow (3) on semilog paper, temperatures against time, and show a remarkable straight-line relationship, except at the higher and lower temperatures, as shown by figure 2. The curves are so drawn as to pass between the last positive for survival, represented by a cross, and the first negative, by a circle, in the greatest possible number of pairs of observation points. It is to be noted that, contrary to the results recorded by Bigelow with spore-forming bacteria, the curve turns downward at the higher temperatures, showing a tendency for quicker killing than would be indicated if the straight line of the intermediate temperatures were followed. Likewise, contrary to Bigelow's curves with bacteria, the curve turns upward at the lower temperatures, showing a tendency toward slower killing than would be expected from the continuation of the straight line of the intermediate temperatures. All points above the straight line represent, of course, time and temperature combinations that are fatal to the stage of the nematode indicated, and all points below represent combinations at which the organism survives.

Figure 2 also presents similarly the data with *Heterodera radicicola* larvae. Here the straight-line relationship strictly holds only for temperatures between 43° and 47° C. and the times between 3 and 8 minutes. At higher temperatures the killing is quicker and at lower temperatures slower than indicated by the continuation of the straight line of the intermediate temperatures. At the lower temperatures, between 40° and 43° C. another straight line exists, connecting all 4 points of observation and showing by its more gradual slope the slower killing than at the intermediate temperatures, with both eggs and larvae.

TABLE 2.—*Percentage survival of Heterodera radicicola eggs in egg masses at different temperatures and different periods of time (average of 6 readings)*

Temperature, °C.	Interval between observations	Number of interval after exposure										
		1	2	3	4	5	6	7	8	9	10	11
40	6 hr.	98.65	98.68	73.99	62.79				15.26 (18 hr.)	(24 hr.)	(25 hr.)	(26 hr.)
41	1 hr.	98.71	94.50	90.78			82.27	75.72	36.18	21.07	19.71	18.7
42	30 min.	98.8	88.13	51.13	31.90	5.08	1.19	0.0	0.0			
43	10 "	92.26	79.96	67.54	28.53	27.92	22.07	6.88	5.17	2.73	0.0	
44	5 "	94.11	80.93	63.70	30.67	17.0	3.23	2.28	1.14	0.39	0.0	0.0
45	1 "	98.30	98.29	94.45	93.30	90.70	84.24	81.96	79.33	75.58	71.18	35.72
46	1 "	98.07	85.93	69.58	60.96	53.49	43.87	37.48	34.02	25.34	18.48	11.74
47	1 "	93.65	76.64	57.68	51.71	48.55	34.35	22.59	21.68	16.07	11.33	0.0
48	1 "	73.36	57.18	53.0	29.71	17.06	3.29	0.0	0.0			
49	1 "	56.23	43.22	4.93	2.25	0.0	0.0	0.0				
50	1 "	2.68	2.63	0.25	0.0							
51	10 sec.	54.0					1.0					
52	5 "	97.0	74.0	1.0			0.06					
53	5 "	1.0	0.05				0.02			0.0		
54	1 "	95.0				0.0						
55	1 "	80.0				0.0						
56	1 "			12.0	3.7	0.0						
57	1 "		2.9	0.0								
58	1 "	0.0										

TABLE 2.—(*Continued*)

Temperature °C.	Interval between observations	Number of interval after exposure								
		12	13	14	15	16	17	18	19	20
40	6 hr.	2.77 (27 hr.)	(28 hr.)	(29 hr.)	(30 hr.)	0.65 (31 hr.)	(32 hr.)	(33 hr.)	(34 hr.)	0.0 (35 hr.)
41	1 hr.	13.88	11.48	9.33	5.80	3.24	1.45	0.97	0.0	0.0
42	30 min.									
43	10 "									
44	5 "	0.0								
45	1 "	19.47	13.73	4.78	0.0					
46	1 "	6.07	2.50	1.79	0.0					
47	1 "	0.0	0.0							
48	1 "									
49	1 "									
50	1 "									
51	10 sec.	0.0								
52	5 "	0.0								
53	5 "									
54	1 "									
55	1 "									
56	1 "									
57	1 "									
58	1 "									

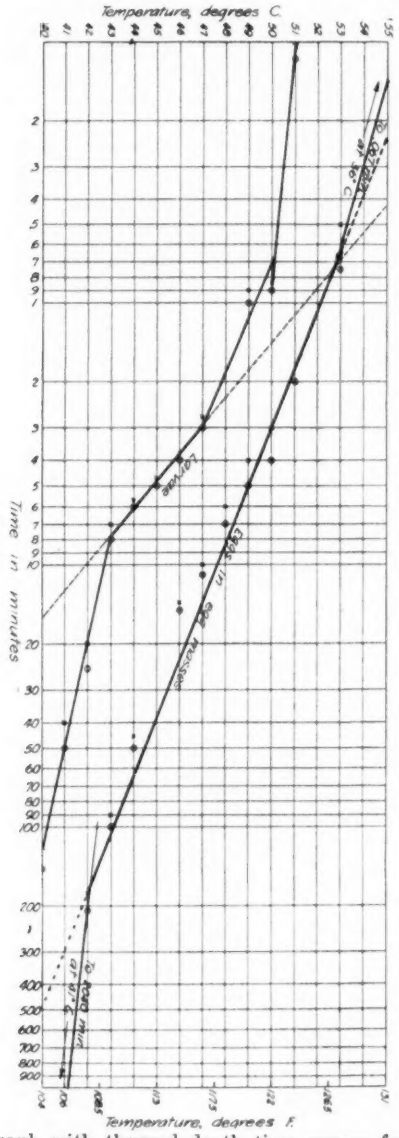

FIG. 2. Semilog graph with thermal death time curves for *Heterodera radicicola* larvae and eggs. Note the relatively straight-line relationship between temperature and time at the intermediate points. All points above the curve represent time and temperature combinations that are fatal to the stage indicated, and all points below represent points at which the nematode survives.

The mathematical theory upon which the use of the semilog paper for thermal death-time curves is based is explained by Buchanan and Fulmer (4, p. 109).

As stated before, special studies were conducted to determine the minimum temperatures at which 10-minute exposures would bring about complete killing, according to practices common in bacteriology. In these studies there were 5 replications of each treatment, 2 egg masses to a tube. Without resorting to tabular form, the results in average survival of larvae for the significant temperatures are as follows: 40° C., 100 per cent; 41° C., 99.4 per cent; 42° C., 36.2 per cent; 43° C., 0 per cent. The lethal point, then, is 42.5° C. ± 0.5°. With egg masses the averages of 6 replications are as follows: 42° C., 99.68 per cent (within the range of the usual controls); 43° C., 92.2 per cent; 44° C., 80.93 per cent; 45° C., 71.18 per cent; 46° C., 18.48 per cent; 47° C., 11.33 per cent; 48° C., 0 per cent. The lethal point is 47.5° C. ± 0.5°, for 10-minute exposure.

CONCLUSIONS AND SUMMARY

This paper constitutes the results of carefully conducted experiments with *Heterodera radicicola* larvae and eggs exposed to direct hot-water treatment at a wide range of temperatures; and, from them, exact data are recorded on the percentage of survival of these stages of the nematode at different temperatures and periods of time and the minimum periods required for complete killing at each of the lethal temperatures. Tables 1 and 2, giving the above information in detail, are here summarized as to the latter point only. The minimum periods of time required for killing the larvae are for the different temperatures in degrees Centigrade: at 40°, 2 hours, 7.5 minutes ± 7.5 minutes; 41°, 45 minutes ± 5 minutes; 42°, 22.5 minutes ± 2.5 minutes; 43°, 7.5 minutes ± 0.5 minute; 44°, 5 minutes, 52.5 seconds ± 7.5 seconds; 45°, 4 minutes, 52.5 seconds ± 7.5 seconds; 46°, 3 minutes, 52.5 seconds ± 7.5 seconds; 47°, 2 minutes, 52.5 seconds ± 7.5 seconds; 48°, 57.5 seconds ± 2.5 seconds; 49°, the same; 50°, 52.5 seconds ± 2.5 seconds; 51°, 6.5 seconds ± 0.5 second; 52°, 1.5 seconds ± 0.5 second; 53°, 1 second. The minimum periods for the eggs are: 40°, 4.5 days ± 0.5 day; 41°, 33.5 hours ± 0.5 hour; 42°, 3.25 hours ± 0.25 hour; 43°, 95 minutes ± 5 minutes; 44°, 47.5 minutes ± 2.5 minutes; 45°, 14.5 minutes ± 0.5 minute; 46°, same; 47°, 10.5 minutes ± 0.5 minute; 48°, 6.5 minutes ± 0.5 minute; 49°, 42.5 minutes ± 0.5 minute; 50°, 3.5 minutes ± 0.5 minute; 51°, 1.5 minutes ± 0.5 minute; 52°, 45 seconds ± 15 seconds; 53°, 37.5 seconds ± 7.5 seconds; 54°, 5 seconds ± 2.5 seconds; 55°, same; 56°, 4.5 seconds ± 0.5 second; 57°, 2.5 seconds ± 0.5 second; 58°, 1 second.

These results are arranged in the form of semilog graphs. The temperatures are plotted on the arithmetic scale and time intervals on the logarithmic. At the intermediate points in time and temperature, a very good straight-line relationship exists. This enables one to see at a glance the

time and temperature combinations at which killing occurs and those at which some of the nematodes will survive.

These temperatures and time periods are exact for the killing of the 2 stages of the nematode. It is necessary, of course, that the specified temperatures reach the organisms and that they be maintained there for the necessary period before the killing is attained. This information is fundamental for any further studies that may involve the application of temperature for root-knot-nematode control. The lethal temperatures are much lower than those commonly employed in practice when it is desired to destroy nematode infestation in the soil or in dormant plant tissues. It will continue to be necessary to employ higher temperatures than those specified in order to obtain penetration of the necessary temperatures to the points in the soil or other medium in which the nematodes are actually located.

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THE TOXICITY OF FORMALDEHYDE AND MERCURIC CHLORIDE SOLUTIONS ON VARIOUS SIZES OF SCLEROTIA OF RHIZOCTONIA SOLANI[1]

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During recent years several papers (2, 3, 4, 5) have appeared on the relative fungicidal efficiency of various chemicals used in solution for reducing the Rhizoctonia disease of potatoes. The relative superiority of these was based primarily on the amount of sclerotia on the tubers of the new crop produced under ordinary field conditions. Melhus, Gilman, and Kendrick (5), also Gloyer (2), formed their conclusions partly by culturing the treated sclerotia and partly by the amount of sclerotia on the new tubers. Obviously the plate-culture test should settle the relative merits of each treatment in killing the sclerotia. With regard to residual effect from formaldehyde or mercuric chloride solutions in protecting the new plantlet, this would be exceedingly difficult to demonstrate, especially in old cultivated soils.

However, as far as could be ascertained, the relation of the size of the sclerotia to the requisite lethal period has not been studied. This would seem important, inasmuch as the size as well as the texture of these bodies varies greatly under various conditions. They may be friable and easily killed or so compact and hard that successful penetration is greatly retarded.

MATERIAL AND METHODS

The sclerotia were classified into sizes, *viz.*, small, medium, and large, and samples indicating the size range for each class mounted on a chart for guidance. Each size varied in flat diameter and thickness, respectively, as follows: Small, about 1.5 mm. by 0.2–0.4 mm.; medium, about 2.5 mm. by 0.5–0.7 mm.; large, about 3.5 mm. by 0.8–1.5 mm. Extra large or extra small or very fragile sclerotia were avoided as far as possible. The treatments included cold formaldehyde solutions, strengths 1–120, 1–160, 1–200, and 1–240; cold mercuric chloride, strength 1–834, and mercuric chloride, strength 1–500 acidulated, with 1 per cent by volume of hydrochloric acid, as recommended by Leach, Johnson, and Parsons (4).

[1] A contribution from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, cooperating with the Department of Field Crops, University of Alberta.

[2] Plant Pathologist in Charge and District Potato Inspector, respectively.

Tubers with sclerotia of various sizes were treated, always in a fresh solution, without preliminary wetting or cleaning, for different periods as required, then covered, and allowed to dry. The drying period required about 3 hours, which is comparable to usual practice on the farm. The sclerotia were then plated on potato-dextrose agar made acid by mixing in the cooling medium, 3 per cent by volume of 5 per cent lactic acid. This allowed the Rhizoctonia fungus to grow satisfactorily but controlled the bacteria. In fact, it was found essential to acidify the agar to do reliable work. Each size of sclerotia was plated separately. Readings were made at intervals up to 4 days, which time was sufficient to indicate whether the bodies were killed.

The sclerotia were found by repeated tests to be almost 100 per cent viable, thus making unnecessary controls for each treatment. Moreover, it was found that the growth of bacteria and molds from untreated sclerotia produces unsatisfactory results. The mercuric chloride and hydrochloric acid used was "Bakers C.P. analysed." The formalin was a commercial brand found by chemical analysis in our laboratory to contain 37.67 per cent formaldehyde. The results throughout are given to the nearest decimal. The relative humidity of the room in which the potatoes for the formaldehyde tests were stored was about 85 per cent and that of the room in which the stock for the mercuric chloride tests was kept, about 56 per cent.

FORMALDEHYDE AND MERCURIC CHLORIDE TREATMENT

Formaldehyde. The results in table 1 and figure 1 indicate the general trend of the lethal period required for the small, medium, and large sclerotia in the 4 strengths of cold formaldehyde solution mentioned. Although time intervals of 10 minutes were employed, only the results at 30-minute periods are given in the table. At strength 1–120, all the small sclerotia were killed in 90 minutes, the midsize ones, in 180 minutes, and those of large size, in 270 minutes. At strength 1–160, the lethal periods for the small, mid-, and large sizes were 120, 240, and 360 minutes, respectively, and in strength 1–200 were 120, 300, and 400 minutes, respectively. In strength 1–240, the small bodies were all dead in 150 minutes, the midsize ones in 390 minutes, while about 1 per cent of the large size were still viable after being immersed for 480 minutes. Further, it should be noted that in a strength of 1–240, 19 per cent of the midsize bodies and 56 per cent of the large size were viable at the end of 2 hours. Apparently cold formaldehyde, strength 1–240 (1 pt. to 30 gal.) is effective only for small sclerotia or where tubers are relatively clean. It is felt that these results would apply to average sclerotia as found in nature in Alberta, although evidence obtained during these studies indicates quite conclusively that the sclerotia

TABLE 1.—*The percentage[a] of small, medium, and large sclerotia of Rhizoctonia solani viable after treatment in various strengths[b] of formaldehyde solution for various periods at 20–25° C.*

Time[c]	Small				Midsize				Large			
	1/120	1/160	1/200	1/240	1/120	1/160	1/200	1/240	1/120	1/160	1/200	1/240
30	2	6	14	21	26	35	54	67	43	57	93	
60	1	2	19	13	16	20	30	42	30	48	66	
90	0	1	3	5	8	14	18	27	26		40	70
120		0	0	2	3	10	13	19	22		31	56
150				0	1	7	9	17	16	19	23	42
180					0	5	7	12	10	13		31
210						2	5		5	8		
240						0	2		2	6		23
270							2	5	0	4	10	20
300							1	3		3		
330							0	2		2	5	9
360								1		0		
390								0			3	5
420											1	3
450											0	2
480												1
510												0

[a] Based on 200–400 sclerotia for small size, 100–150 medium size, and 50–90 sclerotia for large size, and verified by treatments made at intermediate periods of 10 minutes.

[b] Indicated by 1/120, 1/160, 1/200, and 1/240.

[c] Minutes immersed in solution (not presoaked).

on certain lots of potatoes may be harder to kill than those used in these tests.

Melhus, Gilman, and Kendrick (5) obtained 6 per cent of viable sclerotia after treatment for 2 hours in strength 1–120 cold formaldehyde at 26° C. The number of sclerotia used was 100, but no reference was made regarding size or texture of these. At 25° C. and the same time, but strength 1–240, 8 out of 100 sclerotia grew, while, in another lot of 30 planted, none grew. In view of our work, it would seem that their results might be explained partly on the basis of size and texture. It is interesting also to note that Gloyer (2), using cold formaldehyde solution, strength 1–240, for 2 hours, at 17–22° C., obtained 23.8 per cent viable sclerotia out of 327 cultured, and in strength 1–60 at 21° C., for 2 hours, 5 per cent of 60 sclerotia grew. These results are more comparable to those presented in table 1, although Gloyer did not state the size of the sclerotia.

Mercuric chloride. The results from the cold mercuric chloride solution, nonacidulated, strength 1–834, and from the acidulated solutions, strengths

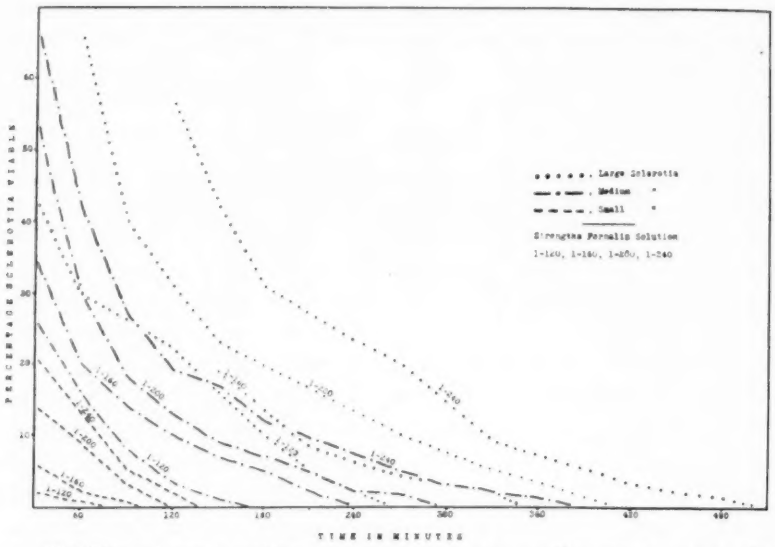

FIG. 1. **Viability of small, medium, and large sclerotia of *Rhizoctonia solani* after immersion in formalin solutions of different strengths for various periods.**

TABLE 2.—*The percentage of sclerotia of Rhizoctonia solani viable after treatment in solutions of mercuric chloride (acid and nonacid) for various periods at 20–25° C.*

Size	Small				Midsize				Large			
Strengths	1–500		1–834		1–500		1–834		1–500		1–834	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Time[b]					(Acidulated[a] solution)							
3	100	0	100	2	100	1	50	28	28	11	25	68
5	100	0	100	0	100	0	50	6	60	8	30	37
8	100	0	100	0	100	0	40	12	69	6	20	10
10							50	7	81	3	33	33
13							60	3	90	2	25	28
15							50	0	65	0	25	20
					(Nonacidulated solution)							
30			150	5			150	37			84	68
45			190	2			33	14			18	62
60			300	0			57	6			49	43
90			200	0			100	3			60	33
120							150	1			80	25
130							135	0			45	16
150							190	0			71	9

[a] By adding 1 per cent by volume hydrochloric acid C.P.

[b] Minutes in solution.

1–500 and 1–834, are presented in table 2. Apparently the cold mercuric chloride solution, nonacidulated, and strength 1–834, is about as effective as the cold formaldehyde solution, strength 1–120. All the small sclerotia were killed in 60 minutes, the midsize ones in 130 minutes, but 9 per cent of the large sclerotia were still viable after 150 minutes. The end point was not determined, but it would likely be about 200 minutes. Thus, where large sclerotia are very scarce, a treatment for 2 hours or even 90 minutes would be effective for practical purposes, but, where they are numerous, between 25 and 30 per cent would still be viable.

Mercuric chloride, acidulated. Cunningham (1), in New Zealand, first discovered that the addition of hydrochloric acid to mercuric chloride solution reduced the time required to kill sclerotia of *Rhizoctonia solani* to about 5 minutes. Leach, Johnson, and Parson (4), as a result of recent work, recommend treatment for 5 minutes in strength 1–500, plus 1 per cent by volume of hydrochloric acid. Considering the results from the acidulated mercuric chloride treatments (Table 2), at strength 1–834 for 5 minutes, none of the small, 6 per cent of the midsize sclerotia and 37 per cent of the large ones grew. After a treatment of 15 minutes, 20 per cent of the large bodies were still viable. The end point for this size was not determined. At strength 1–500, all the small and medium sclerotia were killed in 5 minutes, but 8 per cent of the large ones grew. Fifteen minutes were required to kill all the large ones. Thus, the time strength of acid mercuric chloride (1–500 for 5 minutes), recommended by Leach *et al.*, would be effective for practical purposes, provided large sclerotia were not abundant and that these were not unusually hard to kill. Further, the striking difference in effectiveness between strengths 1–500 and 1–834 emphasizes the advantage of using the former strength instead of the latter one.

Decrease in strength. Leach *et al.*, (4) determined by chemical analysis the loss in hydrochloric acid and mercuric chloride from the solution (strength 1–500 plus 1 per cent HCl) following successive treatments of potatoes. The original solution had 0.2266 per cent of $HgCl_2$ and 0.376 per cent of HCl. After 8 treatments these were 0.1864 and 0.328 per cent, respectively.

It seemed advisable to determine the decrease in effectiveness by culturing the sclerotia from successive lots treated in the same solution. Equal quantities by weight of potatoes bearing sclerotia were immersed, one after the other, in the same solution for the required time (5 minutes for each lot in the acidulated solution or 60 minutes in the nonacidulated one) and the sclerotia plated according to size. Two different lots of potatoes were used for this test: one, lot "A" (same as used throughout this study), from which only the small sclerotia were cultured; and another, lot "B,"

from which all sizes were plated. The sclerotia from lot "B" were firmer in texture and, as indicated by the results in table 3, somewhat harder to kill than those of lot "A."

TABLE 3.—*The decrease in effectiveness of solutions of mercuric chloride as indicated by percentage[a] of sclerotia of Rhizoctonia solani viable after successive treatments*

	Acidulated—5 minutes				Nonacidulated—60 minutes			
Size	Small		Midsize	Large	Small		Midsize	Large
Lot[b]	A	B	B	B	A	B	B	B
Times	1–834	1–500	1–500	1–500	1–834	1–834	1–834	1–834
1	0	0	2	17	0	3	21	62
2	0	0			0			
3	0	0			0			
4	0	0			0			
5	0	2	4	15	1	3	36	73
6	1	1	4	18	1	5	43	77
7	1	4	5	25	3	10	41	77

[a] Based on 100 sclerotia for small and medium sizes and on 50 for large size.

[b] Lot "A," material as used throughout the study. Lot "B," special material, sclerotia firmer than on lot "A."

In the nonacidulated solution, strength 1–834, for 60 minutes, all the small sclerotia on lot "A" were killed up to the 5th, 6th and 7th treatments, in which 1, 1, and 3 per cent, respectively, were viable. On lot "B," 3 per cent of this size grew after the 1st treatment and 5 per cent after the 6th one. At the same strength, 21 per cent of the medium size grew after the 1st treatment, 36, 43, and 41 per cent after the 5th, 6th, and 7th treatments, respectively; while 62 per cent of large sclerotia grew after the 1st treatment and 73, 77, and 77 per cent after the 5th, 6th, and 7th, respectively .

With regard to the acidulated solution, the small sclerotia of lot "A," in strength 1–834, were all killed up to the 6th and 7th times, when 1 per cent grew in each case. The small sclerotia of lot "B," in strength 1–500, were all killed up to the 5th time, when 2 per cent were viable. Two per cent of the medium size of lot "B" grew after the 1st treatment in strength 1–500 and 4, 4, and 5 per cent after the 5th, 6th, and 7th times. As for the large size, 17 per cent of these grew after the 1st time and 15, 18, and 25

per cent after the 5th, 6th, and 7th times. If we may judge on the basis of results obtained from the small sclerotia, indications are quite definite that either the acidulated or the nonacidulated solution may be used at least 5 times without serious reduction in effectiveness.

EFFECT OF TREATMENT ON VITALITY OF SET

Field and laboratory tests were made of the effect of various treatments, mentioned in tables 1 and 2, on the vitality of the set. Part of a lot of Netted Gem potatoes was kept in cold storage so that only very short sprouts started. On the other part, kept in a basement, sprouts upwards of $\frac{1}{2}$ in. long developed. This material was treated as required, $\frac{1}{2}$ of each tuber being planted in sand in the greenhouse and kept moist, the other half in rows in the field, 20 sets per row, 18 in. apart. The following time-strength treatments of formaldehyde were used: 1–120 for 60, 180, and 240 minutes; 1–160 for 80, 220, and 310 minutes; 1–200 for 100, 270, and 390 minutes; and 1–240 for 120, 320, and 470 minutes.

Using the same procedure, the following mercuric chloride treatments were made: nonacidulated, strength 1–834 for 60, 90, and 120 minutes; and acidulated, strength 1–834 for 5, 10, and 15 minutes, and strength 1–500 for 3, 5, and 8 minutes.

Notes on the greenhouse material were taken at emergence and again when the sprouts were about 6 in. high and on the field material at emergence and at blossom time. Briefly stated, the results in all cases, based on observation, were that no detrimental effects of the various treatments on the vitality of the sets could be seen. Yield data taken also showed no significant differences arising from any of the treatments. Obviously, final conclusions cannot be drawn from only 1 year's results.

SIZE OF SCLEROTIA IN NATURE

The results already presented conclusively show that, in determining the effectiveness of any treatment, size of sclerotia is quite as important as time of immersion or strength of the solution. Hence, it is at least interesting to know the proportion of each size commonly occurring in nature. The data in table 4 suggest the percentage of large, medium, and small sclerotia found on 9 representative lots of potatoes grown near Edmonton in 1931, all of which were harvested rather late from soils of relatively high moisture content. If the scurf could have been counted or estimated as "small" on a numerical basis, the percentage of this size would have been increased. It, however, was found that the proportion of small, medium, and large size, as counted, was roughly 13:3:1. Of course, these figures

TABLE 4.—*Relative sizes*[a] *of sclerotia of Rhizoctonia solani on 9 lots of potatoes, 1931 crop, Edmonton, Canada.*

Lot	Bin[b] rating	Tubers counted	Total sclerotia	Small	Midsize	Large	Number scurfy	Date harvested
	Per ct.			*Per ct.*	*Per ct.*	*Per ct.*		
1	50	29	1,206	83	12	5	15	Oct. 1– 7
2	16	19	419	93	6	1	4	" 5– 7
3	4	28	728	80	15	5	8	" 10–16
4	9	14	272	52	38	10	4	
5	25	34	124	92	8	0	7	Sept. 28–30
6	4	20	475	78	17	5	3	Oct. 1
7		24	658	67	22	11	0	
8	1	11	125	73	21	6	1	Oct. 1– 8
9	6	31	725	69	20	11	0	Sept. 23–30
			Average	76.3	17.6	6		

[a] Dimensions of small, midsize, and large approximately the same as used throughout this paper.

[b] The number of tubers out of 200 chosen at random on which any sclerotia occurred.

are only suggestive, but, if further investigation shows this proportion to be approximately normal, due weight can be given to any of the "time-strength-size" treatments recommended for general use.

DISCUSSION

These studies show very clearly that the standard cold formaldehyde solution (1–240 for 2 hours) is not effective for general use in treating tubers where medium and large sclerotia are to be killed. Briefly stated, this treatment would not seem justified on any grounds except when sclerotia are practically absent or limited to very thin scurf. Why a number of the bodies were not killed even after 4–5 hours' soaking was not definitely determined, except that they were always the compact, hard ones. Firm, small selerotia, difficult to kill, were not uncommon. A fairly large proportion of both medium and large sizes were loose in texture and quickly killed. Had the material been presoaked, the effectiveness of all treatments would have been increased materially, but this was not done beyond determining that presoaking is valuable in this connection.

With regard to mercuric chloride, these studies show quite conclusively that a solution of strength 1–834 for 1 hour was about as effective in killing

the small and medium sizes as formaldehyde, strength 1–120 for 2 hours. By adding 1 per cent, by volume, of hydrochloric acid to a mercuric chloride solution, strength 1–500, effective results for practical purposes can be obtained in about 5 minutes, provided large or extra hard sclerotia are not abundant. This fact should be sufficient to recommend its use in place of either the cold formaldehyde or the nonacidulated mercuric chloride solutions for killing sclerotia of *Rhizoctonia solani*.

SUMMARY

The relative effectiveness of various time strengths of cold formaldehyde, nonacidulated, and acidulated mercuric chloride in killing small, medium, and large sclerotia of *Rhizoctonia solani* on the surface of potatoes was determined. The approximate dimensions of these bodies in flat diameter and thickness are given.

In the standard cold formaldehyde solution, strength 1–240, 2 per cent of the sclerotia of small size, 19 per cent of medium size, and 56 per cent of the large size were viable after being immersed for 2 hours. It required 390 minutes to kill all the medium sclerotia and 480 minutes for the large ones. Stronger solutions were increasingly effective for all sizes. The lethal period for the small, medium, and large sizes in strength 1–120 was 90, 180, and 270 minutes, respectively.

The cold mercuric chloride solution, strength 1–834, was more efficient than any of the cold formaldehyde solutions used. The lethal period for the small, medium, and large sizes was about 60, 130, and 150 minutes, respectively, depending upon the texture.

The acidulated mercuric chloride solution, strength 1–500 plus 1 per cent by volume of hydrochloric acid, killed all the small sclerotia in 3 minutes and the medium size in 5 minutes. Eight per cent of the large size were still viable after 5 minutes and 2 per cent after 13 minutes. Treatment in strength 1–834 killed the small sclerotia, but a rather high percentage of the medium and large sizes grew.

Evidence from plating treated sclerotia showed that where small or medium sclerotia are concerned, the solution of either acidulated or nonacidulated mercuric chloride was effective up to the 5th successive treatment and, for practical purposes, probably up to the 8th by extending the time.

The variability in texture of the sclerotia on any lot of potatoes or among different lots is often very marked, making necessary different lethal periods in a given solution.

Under the conditions of the test (one season) none of the following time-strength treatments used perceptibly weakened the vitality of the set:

Cold formaldehyde, strength 1–120 up to 240 minutes; 1–160 up to 310 minutes; 1–200 up to 390 minutes; and 1–240 up to 470 minutes; cold mercuric chloride, strength 1–834 up to 120 minutes; and acidulated mercuric chloride, strengths 1–500 and 1–834 up to 8 and 15 minutes, respectively.

From counts made on 9 representative lots of potatoes the proportion of small, medium, and large sclerotia was 13 : 3 : 1.

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INTUMESCENCES ON LEAVES OF EUCALYPTUS CORNUTA, E. COCCIFERA, HIERACIUM VENOSUM, MITCHELLA REPENS, AND THURBERIA THESPESIOIDES[1]

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Intumescences have been reported on a number of plants since Sorauer (8) first applied that name to the masses of cells that appear under certain conditions on leaves, twigs, and even on roots. These outgrowths are usually rather small, and, since, in most instances they are probably not greatly detrimental to the plants, they are often overlooked. The number of species known to produce such abnormalities is, however, increasing from year to year. The writer became interested in intumescences on leaves of poplar some years ago (5) and since that time has noticed their appearance on several plants that have not hitherto been known to produce them.

This paper deals with intumescences on leaves of *Eucalyptus cornuta* Labill., *E. coccifera* Hook., *Hieracium venosum* L., *Mitchella repens* L., and *Thurberia thespesioides* A. Gray.

Eucalyptus cornuta. Several species of Eucalyptus have been reported to produce these abnormalities, but *E. cornuta* has not been named among them. A number of plants of this species are growing in the greenhouses of the Botanical Gardens of the University of Michigan, and very small green intumescences have been seen on all of these. They occur on both the petioles and the blades of the leaves but have not been found on any other organs of the plants. They appear on both surfaces of the leaves, and are not, so far as can be determined, more numerous on one surface than on the other. On the petioles they seem to be scattered at random, without reference to upper or lower surface. They have not been found in great abundance on any leaf; at least not as compared with the numbers that have been seen on leaves of poplars, but 50 or 60 may be developed on one surface of a given leaf.

So far as the time of development goes, it can be said with certainty that they appear only on the young leaves before they are half grown and do not increase in number after that time, although they may persist until the leaves have reached full size. Nothing is yet known of the causes of these excrescences, and no experiments have yet been performed to determine them. Pieces of leaves bearing the intumescences were cut out, killed, imbedded, sectioned, and stained in the usual manner. No difficulties of

[1] Paper No. 381 from the Department of Botany, University of Michigan.

technique were encountered, so it is unnecessary to discuss methods in detail.

It was found that some outgrowths were confined to the epidermis and so were scarcely to be detected by the naked eye. The cells of the upper epidermis were involved in some abnormalities; in others the lower epi-

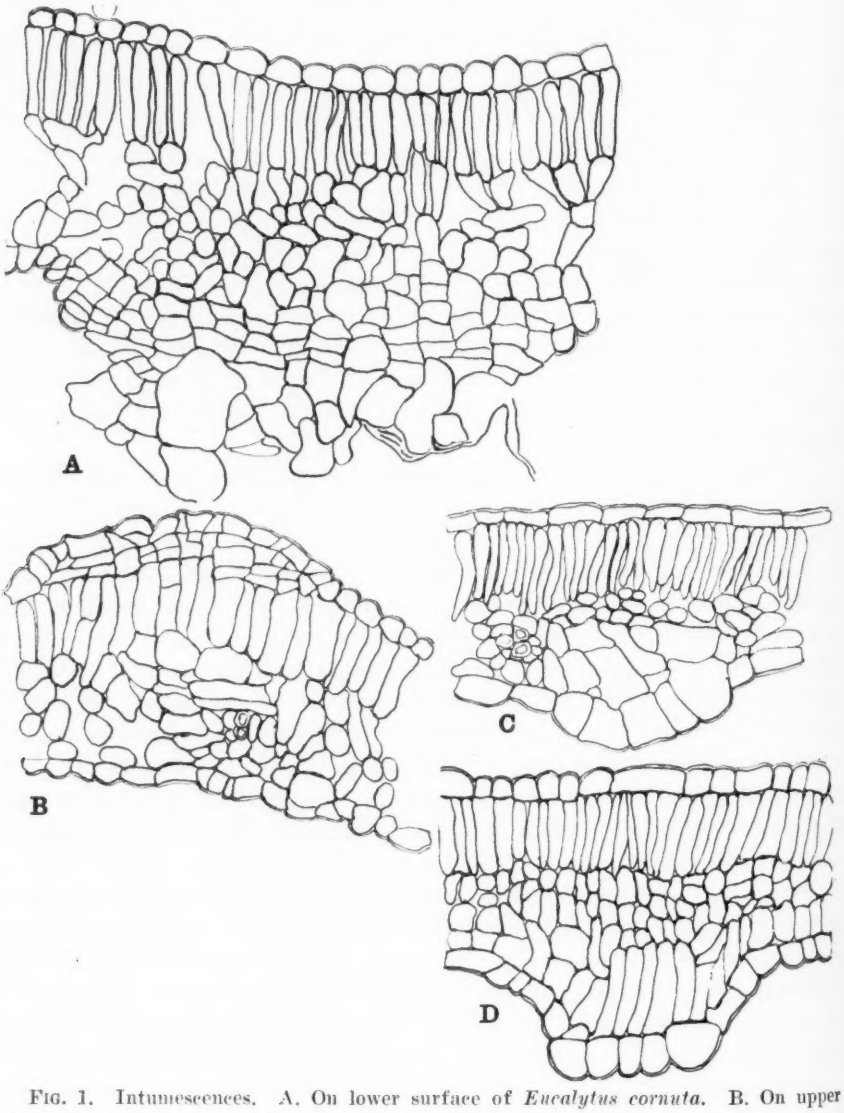

FIG. 1. Intumescences. A. On lower surface of *Eucalytus cornuta*. B. On upper surface of *E. cornuta*. C. Young one on lower surface of *E. cornuta*. D. On lower surface of *Thurberia thespesioides*.

dermis was the seat of the disturbance. Sometimes, the cells are greatly enlarged in all dimensions but have not divided, but, in other instances, they have divided once or twice with a resulting increase in volume.

Outgrowths on the upper surface that extend beyond the epidermis have not been found in any case to reach below the palisade layers (Fig. 1, B). The palisade cells may be elongated without any increase in width and without undergoing division, but, among such cells, others may be seen that have swollen in all dimensions without division, while still others have divided, even repeatedly. Usually, the swollen cells are the ones that divide rather than those that are greatly elongated without corresponding increase in width. The elongation of these cells is, as might be expected, at right angles to the surface of the leaf, since the pressure of other cells on their sides prevents so great an increase in the direction parallel to the surface.

In has been mentioned that the intumescences remain green, and in the sections the chloroplasts are seen to be normal, although their number is not proportional to the increase in volume of the cells. The vacuoles of the cells are greatly enlarged, but no other change in the appearance of the cells could be detected. The cell walls appear to be unchanged except for their increase in extent.

The abnormalities that appear on the lower surfaces of the leaves are quite like those on the upper surfaces, except, of course, for the difference in the tissue involved (Fig. 1, B and C). Here, the changes do not extend beyond the spongy parenchyma but they usually affect all the cells of that tissue. One does not find a great elongation of the cells in one dimension, as occurs in the palisade cells, apparently because the looser tissue allows them to expand more readily in all directions. In this tissue the cells may undergo enlargement without division or they may divide once or more. In other respects the changes are very like those of the palisade cells.

One finds the intumescences, then, whether on the upper or the lower surface, made up of an admixture of enlarged cells, cells without enlargement or division, those that have divided without noticeable increase in size, and others that have divided and also undergone extensive swelling. Such growths might be spoken of as a mixed type, since they are neither hypertrophies nor hyperplasias but a conglomeration of both. In cross section they are seen to vary from 5 to 60 cells in extent.

In many of the intumescences there is a beginning of periderm development, which may have its inception in the epidermis itself, or in any of the layers of the mesophyll, depending on the surface on which the overgrowth is developed (Fig. 1, A). Again, the layer of periderm may form in an arc beginning in the epidermis, on one side, and dipping into the mesophyll to the lower layer of the palisade or to the upper layer of spongy

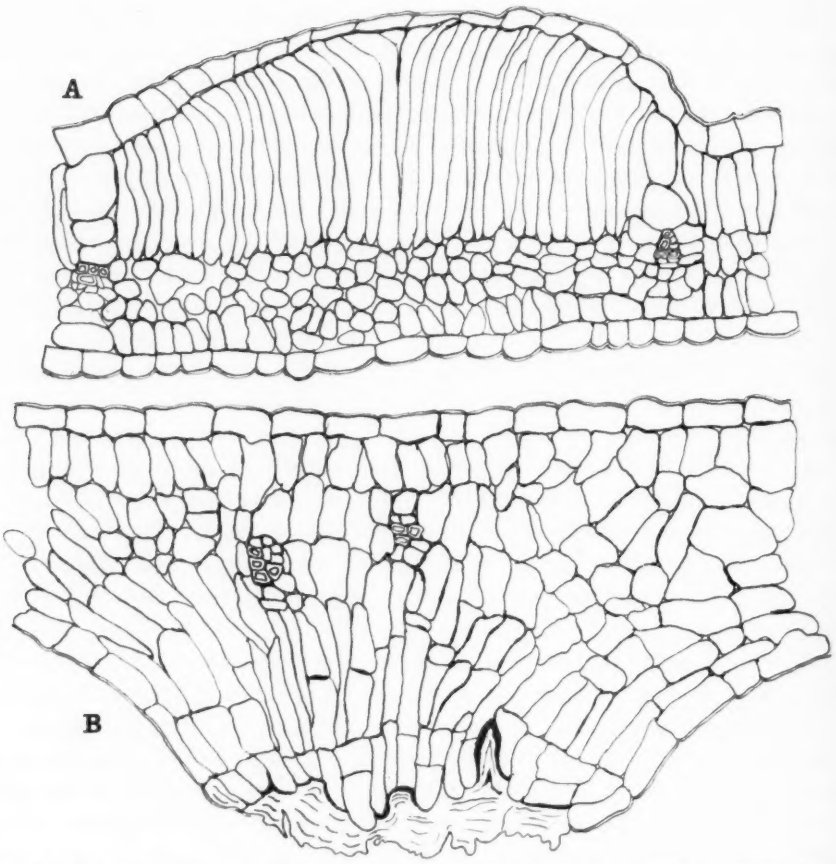

FIG. 2. Intumescences. A. On upper surface of *Thurberia thespesioides*. B. On lower surface of *Hieracium venosum*.

mesophyll, respectively, then passing out to the epidermis, on the other side, so that the intumescence is completely isolated from the remainder of the leaf. Soon after the periderm is formed the cells of the intumescence that lie outside this layer collapse, and the periderm becomes suberized. Thus, the leaf is completely closed against the entrance of fungi and sealed against loss of water.

Eucalyptus coccifera. Sorauer (9) has reported the presence of intumescences in this species but has given no details of their development. Plants of this species, growing alongside those of *E. cornuta* in the Botanical Gardens of the University of Michigan, attracted the attention of the writer because of the presence on the leaves of numerous bright red spots, which were scarcely raised above the surfaces, and did not suggest that they

were of the nature of intumescences. Examination with a microscope, however, indicated such a nature, which was proved by a study of sections of the affected leaves.

The leaves of *Eucalyptus coccifera* are isobilateral, with palisade on both surfaces, although this would not be expected from their position, which is that of ordinary leaves. In general, isobilateral leaves assume a vertical position, and the horizontal arrangement of leaves usually suggests that they are dorsi-ventral as regards their internal structure. In spite of the fact that both sides of the leaves in this species are almost identical, the intumescences are much more abundant on the upper than on the lower surface.

Nothing has been found out as to the cause of these outgrowths, which come near being hyperplasias, since they are made up of cells that are little increased in size over those of the normal leaf. The number of cells, however, is greatly increased in the overgrowths, which, in general, is characteristic of hyperplasias. There is nothing of special note in these growths except the development of periderm, which separates the mature intumescences from the normal parts of the leaf. There is usually one layer of periderm that is very well developed, and that is located in the outer part of the outgrowth. Later on, another layer may be formed near the innermost part of the intumescences, and it is not unusual to find a third layer between these two, which was probably developed before the inner layer. The intumescences that have been studied in this species were mature and were seen to consist of alternate layers of mesophyll cells and periderm. No young stages have been secured for study, so that it is impossible to be sure of the sequence of developments in all cases. All layers of the periderm became suberized.

The bright red of the intumescences seems to depend on the isolation of the mesophyll cells, which contain chlorophyll, from the interior of the leaf, with a consequent accumulation of sugar in the isolated cells, and an extensive development of anthocyanin. The red color indicates an acid reaction in the cells, which has been found by Harvey (1) in intumescences on Ricinus, beet, and Bryophyllum leaves.

Hieracium venosum. The abnormalities on this plant were produced in response to injections of water and of weak solutions of ammonia. Smith (10) found that injections of ammonia and other chemicals caused abnormal growths in *Ricinus communis* L. and other plants. In imitation of his methods, the writer made similar injections into plants of several different species, without result except in *H. venosum*. Some time after the treatment it was found that low intumescences had developed around the site of the injection in a number of the leaves. They appeared both in the

plants injected with ammonia and in those that had received injections of water only. Hence, it appears most likely that the growths are due rather to the injury caused by the insertion of the needle than to the substance injected. All the injections were made through the lower epidermis, and all the intumescences were found on the lower surfaces of the affected leaves and in a ring immediately around each of the wounds.

Sections of intumescences reveal a great increase in cell number in the spongy mesophyll, which is the only tissue affected, if we exclude the epidermis, which is pushed out at first and later is ruptured but not otherwise affected. The cells show considerable swelling and enlargement of the vacuoles but evidence no important changes in the structure of their walls. The chloroplasts are more widely distributed than in the normal cells but are not distorted. As in all other intumescences studied by the writer, the strongest growth lies near the veins. Often two or more small veins are involved.

Periderm development occurs in these outgrowths in the outer layers of cells. A beginning of this is shown in fig. 2, B. When periderm formation has been completed the leaf is again sealed against water loss and invasion of parasitic organisms, and the leaf completes its normal length of life without further effect of the abnormalities.

Mitchella repens. In a previous paper (4), an account was given of some experiments on leaves of *M. repens* in an attempt to determine the source of the water supply of the epidermis. In some of these experiments the lower epidermis was separated from the spongy mesophyll, and in some others the epidermis was removed from the lower surface of the leaf, which was kept afterward in a moist chamber to prevent death of the cells by desiccation. Under these circumstances the leaves threw out masses of cells from the spongy mesophyll, which had thus been relieved of pressure. All appearances would indicate that these growths were intumescences. Sections were made of representative specimens the study of which showed that masses of cells had been formed by division of the cells of the mesophyll nearest the lower epidermis. Some swelling of the cells had occurred but not enough to account for the rise of intumescences. Numerous divisions had taken place and the number of cells in an intumescence was greatly increased over that found in a section of the normal leaf. Periderm formation was found neither in the growths under the lifted epidermis nor in the leaves from which the lower epidermis had been removed. Both these situations would offer rather unsatisfactory conditions for the growth of periderm if Massart's (6) idea that cicatrization does not occur in wounds that are kept in a moist atmosphere is correct. Rodger (7), however, has shown that too much emphasis must not be put on this contention, for peri-

derm may be formed in parts of plants submerged from the time of wounding. It is still possible that the presence of a very moist atmosphere may inhibit periderm formation, where the actual water as a liquid does not.

Thurberia thespesioides. A few plants of *T. thespesioides* have been kept in the greenhouses at the Botanical Gardens for several years. For 3 successive years it has been observed that the leaves were covered with small intumescences. On some leaves little surface has been left free and normal, so that one unfamiliar with the plant might almost suppose the leaves were naturally rugose, instead of smooth, as they really are. A few leaflets could always be found that showed the normal appearance. Thus far, nothing is known of the cause of the growths on this plant.

Examination of sections of the leaves shows that the abnormalities form on either surface, though they are most numerous on the upper side. Those that appear on the upper side involve only the single layer of palisade cells and do not extend into the spongy mesophyll. The palisade layer is not affected by the outgrowths on the lower surface, which are confined to the spongy layers. The epidermal cells may or may not increase in size, but they never divide. The epidermis may be stretched to allow room for the enlarged cells inside it, but it is not often torn open, as it is in many intumescences on other plants. The relation of veins to intumescences has been noted before in this paper and also in a previous paper by the writer (5). In this plant, too, the growths are best developed near the veins.

The intumescences of Thurberia are due largely to the elongation of the cells, although some cell divisions take place. In some parts of the leaves one finds cells that have increased in width as well as in length, but by far the greater number of elongated cells show little or no widening, and, as a result, they come finally to have a most abnormal shape. (Figs. 1, D, and 2, A.) The chloroplasts of the cells are not injured and the nuclei appear normal.

Periderm seems to form in those cases where the epidermis is split open, but, as has been said, most of the intumescences never rupture the epidermis. Hence, the outgrowths cause no particular harm to the leaves on which they develop, although they remain until the leaves fall.

DISCUSSION

The intumescences described on the foregoing pages are all, with the exception of those on Thurberia, of a type that may be called "mixed," since they combine the characteristics of hypertrophies and of hyperplasias; that is, they are formed by the swelling and division of the mesophyll cells. In Thurberia the outgrowths may show a few cell divisions, but they are produced mostly by the swelling of the cells of the regular

layers of the mesophyll. Both of these types are known from the descriptions of other authors.

Küster (2) would probably not consider the abnormalities on *Hieracium venosum* and on *Mitchella repens* intumescences at all but would refer them to the category of wound reactions. It does not seem that the distinction should be pushed too far, because, after all, such growths as Küster considers true intumescences, those he has described on *Populus tremula* L., for instance, while they are not caused by wounding, probably are due to abnormal environmental conditions that may not be different in their final effect. To the writer it seems that the form of growth is a better criterion for the decision as to what name shall be applied to it than is the cause of its inception.

The development of anthocyanin, seen in the intumescences on *Eucalyptus coccifera*, has not been observed by the writer in any other plant, nor has mention been made of it by any other author so far as is known to him. Such a production of pigment is a very common occurrence in galls and also in the outgrowths of epidermal cells caused by attacks of species of Eriophyes (3). From the similarity of such growths to intumescences, it is rather surprising that the latter do not develop anthocyanin more frequently.

The development of periderm in the intumescences on *Eucalyptus coccifera* is more extensive than that observed by the writer in any other such growth or seen in the illustrations of any other intumescence on a leaf. Presumably the periderm in intumescences is dependent on the facility of periderm formation possessed by the leaf in general and shown in any type of wound on that leaf. Wylie (11) and Rodger (7) have shown that plants possess the power of periderm formation in varying degrees, and one might predict the different manifestations of this power found in different intumescences. The age of a given intumescence also has much to do with periderm formation, and it is likely that some intumescences have not been observed in sufficiently mature stages to reveal its presence.

SUMMARY

Intumescences are described on leaves of *Eucalyptus cornuta*, *E. coccifera*, *Hieracium venosum*, *Mitchella repens*, and *Thurberia thespesioides*.

Extensive cell division occurs in the formation of the outgrowths in the first 4 species named above, but the outgrowths on Thurberia show rather few cell divisions.

The outgrowths show extensive swelling of cells in all the species concerned, with large increases in the size of the vacuoles.

Periderm is formed in all the outgrowths except those on *M. repens*. In *E. coccifera* it is especially well developed.

Anthocyanin is formed in the intumescences on *E. coccifera*, which causes them to appear as red spots on the leaves.

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RESISTANCE OF SOME OF THE CULTIVATED SPECIES OF ALLIUM TO PINK ROOT (PHOMA TERRESTRIS)

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INTRODUCTION

Pink root, recently shown by Hansen (1, 2, 3) to be caused by *Phoma terrestris* Hansen, is a major disease of the onion in California. It is often found in fields growing onions for the first time, but, with continued culture in infested soil, losses ranging from 50 to 96 per cent have been reported. As the causal organism is a soil-inhabiting parasite, the most logical means of control seems to be the development of resistant strains. With this need in mind, we are now seeking resistant species of Allium or resistant varieties of *A. cepa* for hybridizing with those susceptible varieties that are important commercially, and we are selecting for resistant individuals within the susceptible varieties now extensively used in California. The marked resistance manifested by several species of Allium justifies the publication of our results to date.

HISTORICAL

Published data on resistance to onion pink root are somewhat complicated because several distinct organisms are reported as causing the disease. Taubenhaus (6, 7, 8) attributed pink root to an organism that he named *Fusarium malli* Taub., and in all his work on resistance of varieties and species he assumed pink root to be a fusarial disease. Sideris (4, 5) reported several species of Fusarium to be capable of inducing the disease. Hansen (3), however, found that onions did not become infected when inoculated with *F. malli* and stated in his paper that L. F. Alexander, of the University of Wisconsin, had obtained similar results.

Taubenhaus (8) planted the onion varieties Yellow Dutch, Yellow Danvers, Australian Brown, Large Red Globe, Large Red Wethersfield, Large White Globe, White Portugal, and Extra Early Red on infested soil and found all highly susceptible except the last mentioned variety. He thought that Extra Early Red was perhaps, in reality, Red Bermuda, which he stated was fairly resistant, as were other types of Bermuda onions, extensively grown in Texas. He further reported that the "multiplier" varieties of onions, as well as shallot and the Mexican and Italian garlic varieties, were all highly susceptible. No infection was noted on Narcissus, tulip, Funkia, Iris, Freesia, or Lilium when grown in infested soil. Hansen (3) isolated Phoma from cowpeas, *Vigna sinensis;* Lima beans, *Phaseolus limensis;* and potatoes, *Solanum tuberosum.*

We consider that pink root is caused by the species of Phoma named by Hansen, for the disease had developed in epiphytotic form when a culture of *P. terrestris* supplied us by Hansen was added to steamed soil in which onions (from disinfected seed) were growing.

METHODS AND MATERIAL

At University Farm, Davis, pink root has become particularly severe in a small plot that has been continuously planted to onions since 1925. All our tests, except when otherwise specified, have been conducted either in this infested plot or with the same soil in the greenhouse or cold frame. We know this soil to be uniformly infested because, under favorable environmental conditions, approximately 100 per cent infection develops on such varieties as Australian Brown and Yellow Globe Danvers. The methods of irrigation and cultivation are, furthermore, conducive to uniform distribution of the pathogen in the soil. Unless otherwise specified, the trials have been replicated so as to correct for possible heterogeneity of the soil. The uniformity of infection in the individual replications of the same variety gives further evidence of uniform infestation.

The species of the genus Allium that we have tested are indicated below. The commercial varieties Yellow Globe Danvers and Sweet Spanish, now known to be, respectively, highly susceptible and slightly resistant, have been used as checks in measuring the resistance among the various species of Allium. Garlic, *A. sativum*, is now extensively grown in California and Louisiana. Although leek, *A. porrum*, is not widely grown in the United States, some is produced in California. Shallots, *A. ascalonicum*, are a popular crop in certain districts of the South, especially in Louisiana. Chives, *A. schoenoprasum*, are raised only on a small scale in America. A type of *A. fistulosum*, called Nebuka by the Japanese and grown by them as a green onion, makes a rapid growth in early spring, forms no bulb, and may possibly replace shallots in some districts (Fig. 1).

Relative resistance to pink root has been measured in one of several ways. When plants were grown in infested soil in the greenhouse or in cold frames, the root systems were examined to obtain actual counts of diseased plants. Such procedure was also possible when seed was sown thickly in the field and the seedling plants were pulled for transplanting. Obviously, when leaf symptoms or yields were being determined, root infection could not always be recorded. In the field, when the plants were grown to maturity, resistance was determined by (a) percentage of plants living on successive dates during the growing season, (b) relative vigor of living plants, and (c) average weight of the mature bulbs in bulb-forming varieties or species.

FIG. 1. Nebuka, *Allium fistulosum*, which is resistant to attack by the pink-root organism.

RESULTS

On March 15, 1928, green plants of the varieties Yellow Danvers Flat, Southport Yellow Globe, White Portugal, Australian Brown, Red Wethersfield, Southport Red Globe, Ebenezer, Yellow Globe Danvers, California Early Red, Italian Tripoli, and Valencia were planted in infested soil in the field. All the bulbs except Valencia, when dug on July 17, were found so severely infected with pink root that their average weight was 48.4 gm.

or less, while that of Valencia was 81.6 gm. Average bulb weights for the remaining varieties were as follows: Yellow Danvers Flat, 26.5 gm.; Southport Yellow Globe, 25.5; White Portugal, 25.3; Red Wethersfield, 45.8; Southport Red Globe, 35.9; Ebenezer, 36.3; Yellow Globe Danvers, 39.5; California Early Red, 48.4; and Italian Tripoli, 37.1 gm. Relative bulb size cannot always be used as an index of resistance, because of differences in date of maturity and inherent size-determining factors. Later work, nevertheless, has shown that the onions of the Sweet Spanish type consistently mature marketable bulbs in heavily infested soil.

In September, 1929, green plants of Nebuka were set in infested soil in the field. On December 31, 1929, dry sets of garlic, seed of Giant Musselberg leek, Yellow Globe Danvers, and Sweet Spanish onions were similarly planted. Except the 2 latter varieties, the growing plants were not moved until October, 1930, when all were examined for evidence of pink root. As seed of Yellow Globe Danvers and Sweet Spanish had been planted thickly, the seedlings had to be transplanted in May, 1930. At that time, infection of these 2 varieties was 98.2 and 85.1 per cent, respectively. Although 85.1 per cent of the Sweet Spanish plants were infected, many manifested only a mild attack, with a small lesion on 1 or 2 roots. Infection was more complete and severe on Yellow Globe Danvers, for, out of 1,407 plants examined, only 121 were considered sufficiently free of infection to transplant. Sweet Spanish was more tolerant of the disease than was Yellow Globe Danvers.

The results indicated that of the 6 species tested, 4, namely, *Allium porrum*, *A. schoenoprasum*, the Sweet Spanish variety of *A. cepa*, and the Nebuka type of *A. fistulosum*, exhibited marked tolerance of the disease, *Allium sativum* and *A. ascalonicum* were extremely susceptible; and the Yellow Globe Danvers variety of *A. cepa*, although apparently less susceptible than *A. sativum*, proved not to be so when relative plant vigor and percentage of plants severely infected were considered. Although 50 per cent of the Yellow Globe Danvers plants were living on August 25 (as measured by green leaf tissue), many of these made relatively little growth after July 8. New roots, apparently, formed just fast enough, after the older ones became infected, to keep the plants alive but not fast enough to allow normal growth. Conversely, though 96 per cent of the Sweet Spanish plants were living on August 25, not all showed the same relative vigor. Many had considerably reduced leaf area but were much more vigorous than Yellow Globe Danvers. When the bulbs were dug in September, infection of these 2 varieties, as manifested by root symptoms, was almost 100 per cent; but the Sweet Spanish had many more living uninfected roots than did the Yellow Globe Danvers bulbs. The average bulb weight of

these 2 varieties was 76.8 and 28.4 gm., respectively. Several individual bulbs of Sweet Spanish weighed as much as 280 gm., while the largest bulb of Yellow Globe Danvers weighed only 60.

Garlic and shallot were extremely susceptible. Although no complete data were taken on bulb weight of these species, an examination of the roots on August 30 showed almost 100 per cent severe infection.

Chives and leek were markedly resistant. Pink root was perhaps not severe enough on any plants of these 2 species to affect normal growth seriously. Root lesions were usually small, and very few roots showed complete infection. Similar results were secured in the greenhouse during the winter of 1930–31, when 402 chives plants were grown adjacent to 832 Yellow Danvers Flat in infested soil. The relative pink root infection of these 2 varieties was 7.9 and 94.5 per cent, respectively. Though infection on Yellow Globe Danvers was extremely severe, only very mild infection was noted on the chives roots—probably insufficient to interfere with normal growth.

Nebuka exhibited marked resistance. None of the plants died; and the roots, when examined for infection at the end of the growing season, were found only slightly damaged. This type, which grows perennially, has been propagated continuously in infested soil since September, 1929, without the slightest sign of being damaged by the disease. A similar statement may be made for chives and leek, although they have not been propagated so long in infested soil as has Nebuka.

Our next trial was conducted with infested soil in 5-in. pots set in a coldframe. The varieties used were Southport Yellow Globe, White Portugal, Sweet Spanish, Australian Brown, Prizetaker, Southport White Globe, Red Wethersfield, Southport Red Globe, Ebenezer, Italian Red, Yellow Globe Danvers, Ailsa Craig, Extra Early Red Flat, Michigan Yellow Globe, Ohio Yellow Globe, Valencia, Early Medium Yellow, leek, and Nebuka. Seed of these varieties and dry bulbs of garlic and shallots were planted on February 19, 1930. Observations were made on plant size and vigor until May 15, when the plants were pulled, weighed, counted, and examined for infection. The results (Table 1) indicate the percentage of plants moderately and severely infected as well as the calculated average weight per 100 green plants of those infected and those free of disease. These data indicate that leek and Nebuka were extremely resistant and that, of the 17 onion varieties tested, the Sweet Spanish types (Valencia, Sweet Spanish, and Prizetaker) were less susceptible than the remaining 14 varieties. Garlic and shallots were extremely susceptible.

Further tests were conducted in the field in 1930, when Nebuka, leek, garlic, shallots, and several varieties of *Allium cepa* were compared in

TABLE 1.—*Relative susceptibility of leek, Nebuka, and 17 varieties of onions to pink root, as manifested by seedlings grown in infested soil. Plants 85 days old*

Variety	Number of plants tested	Percentage of plants infected			Calculated weight per 100 plants in gm.		
		Severely	Moderately	Total	Severely infected	Moderately infected	Disease-free
Southport Yellow Globe	252	70.0	22.0	92.0	26	83	140
White Portugal	303	10.0	74.0	84.0	10	19	25
Sweet Spanish	952	7.4	66.4	73.8	20	32	39
Australian Brown	827	46.7	49.1	95.8	15	33	52
Prizetaker	307	5.0	64.0	69.0	17	33	98
Southport White Globe	601	31.5	51.5	83.0	18	38	68
Red Wethersfield	527	7.6	75.9	83.5	17	33	76
Southport Red Globe	498	10.6	79.9	90.5	19	42	61
Ebenezer	637	11.1	71.8	82.9	14	24	43
Italian Red	333	14.0	76.0	90.0	12	31	49
Yellow Globe Danvers	623	20.4	72.5	92.9	15	31	38
Ailsa Craig	305	20.0	79.0	99.0	11	36	205
Extra Early Red Flat	247	11.0	81.0	92.0	15	29	50
Michigan Yellow Globe	380	16.0	81.0	97.0	15	27	42
Ohio Yellow Globe	683	21.9	72.6	94.5	13	31	50
Valencia	336	4.0	58.0	62.0	11	26	57
Early Medium Yellow	233	0.0	71.0	71.0	....	35	42
Leek	81	0.0	2.0	2.0	....	20	38
Nebuka	184	0.0	3.0	3.0	....	....	....
Shallots	24	26.9	63.2	90.1	....	....	....
Garlic	24	20.3	71.1	91.4	....	....	....

infested soil. The onion varieties planted were Yellow Danvers Flat, Southport Yellow Globe, White Portugal, Australian Brown, Southport White Globe, Red Wethersfield, Southport Red Globe, Ebenezer, Italian Red, Yellow Globe Danvers, Early Medium Yellow, Yellow Strasburg, Ohio Yellow Globe, Valencia, Sweet Spanish, and Prizetaker. Green plants grown for several weeks in disease-free soil in a cold frame were transplanted into an infested area in the field on May 15. Observations were made regarding percentage of living plants and relative vigor during the growing season; and, as the varieties matured, bulb size and percentage of plants producing bulbs were determined. Summarized data covering this test (Table 2) show that at maturity, in infested soil, those varieties of the Sweet Spanish type produced significantly larger bulbs than did the other varieties. Evidently, furthermore, Nebuka and leek were more resistant than any varieties of *A. cepa* used. No data are given for garlic and shallots in this test, but the plants died well in advance of the normal date of maturity, the bulbs were stunted, and much of the root system was destroyed by the pink-root pathogen.

TABLE 2.—*Response of some species of Allium and of 15 varieties of A. cepa to pink root when green plants produced in disease-free soil were transplanted into infested soil in the field on May 15, 1930, at Davis, California.*

Variety	Number of plants	Percentage of plants living		Relative vigor[a]		Percentage bulbing	Average bulb weight gm.
		June 18	July 19	June 18	July 19		
Yellow Danvers Flat	75	92.0	66.6	3	2+	85	33.6
Southport Yellow Globe	79	91.1	84.8	3	3	93	30.8
White Portugal	79	96.2	89.9	3	3–	94	26.1
Australian Brown	80	88.7	62.5	2	2–	87	18.3
Southport White Globe	80	98.7	85.0	4–	3	90	39.2
Southport Red Globe	79	96.1	81.1	3	3–	94	25.2
Ebenezer	79	91.1	66.1	2	2	98	16.9
Italian Red	80	90.0	82.5	2+	2+	87	30.8
Yellow Globe Danvers	79	87.4	79.8	3–	3–	85	24.4
Early Medium Yellow	80	96.3	85.0	2+	2+	94	22.4
Yellow Strasburg	158	94.3	75.1	3–	2	82	24.4
Ohio Yellow Globe	156	95.1	74.2	2+	2	91	22.9
Sweet Spanish	237	98.9	97.1	4	4–	98	61.6
Valencia	77	98.2	96.1	4	3+	99	58.8
Prizetaker	79	97.1	96.0	4	3+	98	64.4
Nebuka	45	100.0	100.0	4	4	.....	.....
Leek	45	100.0	100.0	4	4	.....	.....

[a] Indicated progressively from 0 (plants dead) to 4 (most vigorous).

The final test was conducted in the greenhouse with Yellow Globe Danvers (susceptible) and Nebuka (resistant) in soils that varied in degree of infestation. Steamed and field-infested soils were used as checks on infested soil that had been diluted with steamed soil and placed in separate compartments of a bench in the greenhouse. The dilutions were 1:10, 1:5, and 1:1 of infested with steamed soil. Seed was planted on March 24, 1931; and final readings were made on May 26, when the plants were approximately 2 months old.

TABLE 3.—*Relative resistance to pink root of Nebuka and Yellow Globe Danvers when grown in soil that varied in degree of infestation. Data from seedling plants in the greenhouse, 1931.*

Soil infestation	Variety	Number of plants	Percentage free of pink root	Percentage of plants infected			
				Severely	Moderately	Slightly	Total
None (steamed soil)	Danvers	2,519	100.0	0.0	0.0	0.0	0.0
	Nebuka	369	100.0	0.0	0.0	0.0	0.0
1:10[a]	Danvers	2,601	42.7	1.9	21.8	33.6	57.3
	Nebuka	628	89.1	0.0	0.0	10.9	10.9
1:5	Danvers	1,647	26.3	7.9	41.1	24.7	73.7
	Nebuka	589	85.2	0.0	0.0	14.8	14.8
1:1	Danvers	2,270	20.1	22.1	33.9	23.9	79.9
	Nebuka	517	72.3	0.0	0.6	27.1	27.7
1:0 (field infested)	Danvers	1,844	9.4	25.4	34.3	30.9	90.6
	Nebuka	584	71.9	0.0	2.3	25.8	28.1

[a] One part field-infested to 10 parts steamed soil.

The results of this final test (Table 3) indicate further that Nebuka exhibited marked resistance to pink root under conditions made favorable for infection where the entire root system could be examined. Although only 71.9 per cent of the Nebuka plants were entirely disease-free when grown in field-infested soil, almost none of the plants was sufficiently infected to interfere with normal growth. One should note that the percentage of infection increased slightly with increased degree of soil infestation. None of the Nebuka plants was severely infected; 1.4 were moderately and 17.3 per cent slightly infected. When compared with Yellow Globe Danvers, furthermore, Nebuka was markedly resistant to the pink root, regardless of the degree of soil infestation.

SUMMARY

Varieties of 6 species of Allium have been tested for resistance to onion pink root, caused by *Phoma terrestris*. *Allium fistulosum* (Nebuka type), *A. porrum* (leek), [(Giant Musselberg variety)], and *A. schoenoprasum* (chives) have proved extremely resistant. *Allium sativum* (garlic), *A. ascalonicum* (shallot), and most varieties of *A. cepa* (common onion) are extremely susceptible, although the variety Sweet Spanish may be classed as moderately susceptible.

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TUBERCULINA MAXIMA IN WESTERN NORTH AMERICA

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INTRODUCTION

The lilac fungus (*Tuberculina maxima* Rost.) has long been known as a parasite of white-pine blister rust (*Cronartium ribicola* Dietr.) in Europe (8, p. 160; 11) and on that Continent appears to be widely distributed on this host (1, p. 252; 5; 10; 14; 18). Although it is known upon several of our native pitch-pine blister rusts (*Cronartium commandrae* Pk. (16, 17), *C. coleosporiodes* (Diet. & Holw.) Arth., *C. cerebrum* Arth. & Kern (17), *C. harknessii* (Moore) Meinecke, *C. stalactiforme* Arth. & Kern (16), and *C. quercus* Schrt. (9, p. 12), it has not been reported on this Continent upon the white-pine blister rust (10), or upon *C. comptoniae* Arth., the sweet-fern blister rust of pitch pines. This paper reports the discovery, distribution, and observed behavior of *T. maxima* upon these two rusts in western North America.

DISCOVERY, HOSTS, AND DISTRIBUTION

Tuberculina maxima[2] as a parasite of *Cronartium ribicola* was first observed by J. S. Boyce, of the Division of Forest Pathology, on cankers on western white pine (*Pinus monticola* D. Don). It was found on August 18, 1926, at Daisy Lake, British Columbia. At this same place *T. maxima* was found on *C. comptoniae* for the first time on April 25, 1931, when the writer collected it on cankers caused by this rust on lodgepole pine (*P. contorta* Loud.). This appears to be the first record of *T. maxima* on this host in North America.

Following the discovery made by Boyce at Daisy Lake, B. C., in 1926, additional records of the occurrence of *Tuberculina maxima* on *Cronartium ribicola* were made during the fall of that same year. The writer found it at another locality at Daisy Lake on October 16 and also at Brackendale, B. C., on October 18. H. G. Lachmund, of the Division of Forest Pathology, noted it at Owl Creek, B. C., on October 17. Since these observations,

[1] The writer wishes to express his gratitude to E. P. Meinecke, Principal Pathologist, Division of Forest Pathology, U. S. Bureau of Plant Industry, for his kind assistance in supplying some of the literature references and for translations of some of the foreign papers.

[2] This fungus was determined as *Tuberculina maxima* Rost. by Boyce, verified by J. R. Weir, and critically compared with European material by C. von Tubeuf of Germany, who confirmed the previous determinations.

the parasite has been noted in only one additional locality in the coastal section—near Little Blackwater Lake, a few miles west of D'Arcy, B. C., where it was found by the writer on June 19, 1930.

The range of *Tuberculina maxima* on *Cronartium ribicola* as now known is confined to a narrow strip of country bordering the line of the Pacific Great Eastern Railway, beginning at Brackendale, B. C., about 30 miles north of Vancouver, and extending northward for about 60 miles to the vicinity of D'Arcy, B. C. Daisy Lake is about 13 miles and Owl Creek about 45 miles north of Brackendale.[3] On *C. comptoniae* the parasite has been found only at Daisy Lake.

This comparatively limited distribution of *Tuberculina maxima* does not result from limited occurrence of these two western hosts. *Cronartium ribicola* was known to be widely distributed on *Pinus monticola* in both the coastal and interior white-pine belts of British Columbia in 1923 (6). The present known range of this rust on western white pine is from near the northern tip of Vancouver Island in British Columbia to the central Cascade Mountain region of Oregon and from the Pacific Coast eastward nearly to Montana. The main range of *C. comptoniae* on *P. contorta* in western North America as outlined by Lachmund (3) is from Prince Rupert, B. C., southward through the coastal region to the mouth of the Columbia River in Washington. The coastal portion of the range of *C. ribicola* on *P. monticola* is, therefore, fairly comparable to the main range of *C. comptoniae* on *P. contorta*. In this part of the country much intensive research has been conducted on *C. ribicola* during the past 10 years. Considering, therefore, the few localities at which *T. maxima* has been noted in this connection, it seems apparent that the occurrence of the parasite is decidedly limited relative to the range of these two rusts.

PARASITE CONSIDERED INDIGENOUS TO THE REGION

If the lilac fungus were introduced with the white-pine blister rust into this region from Europe, one would expect to find it associated at or near the point where the latter was introduced into western North America at Point Grey, near Vancouver, B. C., in 1910 (2). There is, however, no evidence to this effect. Several blister-rust investigators are known by the

[3] Since this paper went to press *Tuberculina maxima* has been found during the summer on *Cronartium ribicola* in the interior section of British Columbia and southward in the United States. The stations are as follow: Revelstoke, B. C., by E. L. Joy, U. S. D. A., Div. Blister Rust Control, Spokane, Wash., Arrow Park and near Rosebery, B. C., by members of the Div. Forest Pathology, U. S. D. A., Portland, Oregon, and Newman Lake, Wash., by R. K. Pierson, U. S. D. A., Div. Blister Rust Control, Spokane, Wash. *T. maxima* was noted to be rare at all these localities except Arrow Park where it was frequently encountered. All localities are old blister-rust infection areas, the majority originating in 1923 and one (Revelstoke) as early as 1917 or 1918.

writer to have examined the infected pines at this original introduction point, and none of them reported the presence of *Tuberculina maxima*. Furthermore, it has never been noted on infected pines anywhere else in the vicinity of Vancouver. On the other hand, there are good indications that the lilac fungus is indigenous to North America. As has been mentioned, this parasite has been found abundant on *Cronartium comptoniae* on *Pinus contorta* near Daisy Lake, B. C., and is known to occur in the United States on several other Cronartium species.

BEHAVIOR OF TUBERCULINA MAXIMA IN BRITISH COLUMBIA

The parasite attacks both pycnia and aecia of the white-pine blister rust. In British Columbia it has been observed that production of the aeciospores is greatly reduced when the aecia are attacked and that death of the bark over the cankered area often occurs in a few years. Rust-infected bark of stem cankers on trees 3 to 8 in. in diameter when parasitized may become noticeably constricted prior to death of affected parts. In Germany, Tubeuf (15) found by inoculations that *Tuberculina maxima* suppressed formation of aecia and repeatedly led to a dying of the bark. The writer has observed that on aecial fructifications the parasite commonly develops a tuberculate formation.

In British Columbia pycnia may be attacked throughout the entire seasonal period of their production, which is generally from June to September, inclusive. A canker parasitized during the first part of the season may produce pycnia all summer, but the new pycnia are generally attacked soon after their appearance. This attack on the pycnia no doubt reduces the volume of pycnospore production. Spaulding (10) observed that in Europe the pycnospores are not produced in great abundance, partly because of the effect of *Tuberculina maxima*, which reduces locally their production in diseased bark.

Sporulation of the parasite on the pycnial stage of the rust is very abundant under British Columbia conditions, particularly in summer during the main period of pycnial production. At this time, cankers have been observed with their pycnial area completely covered by a thick dust-like layer of the lilac-color spores—a phenomenon that has not been noted in like degree during the period of aeciospore production. A pycnial zone attacked one season usually fails to produce aecia the following year.

All indications are that the pycnial fluid is the principal substratum for the growth and development of the lilac fungus. Field observations to this effect are borne out by the results of a study conducted at the suggestion of the writer by Professor H. P. Barss, Oregon State College, Corvallis, Oregon. He found that an aqueous solution of the pycnial fluid was an excellent medium for the germination of *Tuberculina maxima* spores.

This apparent preference of the parasite for growth on pycnia indicates that its effect on aecial production is indirect. Evidence substantiating this has been obtained. During the spring of the past year, at the time aecial sporulation was in progress, specimens of *Tuberculina maxima* were collected from a parasitized stem canker by shaving off portions of the infected bark with a knife. The cuts in the bark were not quite deep enough to remove the cambial layer. This same canker was examined about a month later, and *T. maxima* was then found to be sporulating profusely on portions of the cut surfaces. This seems to indicate that the parasite is not entirely superficial upon pycnia and aecia but that its mycelium invades the bark well beneath these fructifications on the surface. Otherwise, it would not have been able to sporulate so promptly when the outer bark was removed. If this is the case, it may explain why a pycnial zone attacked one season usually fails to produce aecia the following year. It may also explain why the lilac fungus appears to be a factor in hastening the death of cankered bark, which usually remains alive and sporulating for 3 or 4 years in any particular canker zone after the first appearance of aecia. As has been mentioned, Tubeuf (15) found that cankered bark dies after invasion by *T. maxima*, and this has been a common observation of the writer.

Tuberculina maxima has not been reported in Europe on the uredinia and telia of the white-pine blister rust on species of Ribes,[4] nor has it ever been observed on these stages of the rust in North America.

Observations on the behavior of the lilac fungus on the sweetfern blister rust were confined to the Daisy Lake area and were all made during the season of 1931. The data secured indicate that the action of the parasite on this rust is similar to that on the white-pine blister rust. It attacks both pycnia and aecia but sporulates in greatest abundance on the pycnia, which are produced during summer. The parasite does not appear to be generally destructive to *Cronartium comptoniae*.

IMPORTANCE IN THE CONTROL OF WHITE-PINE BLISTER RUST

At present there is no evidence that under British Columbia conditions the lilac fungus can effectively suppress the white-pine blister rust. Comparing the known extensive range of *Cronartium ribicola* with the limited area in which *Tuberculina maxima* occurs, it is apparent that the latter lacks aggressiveness and is very tardy in the invasion of this host. Examples of this have been observed on several occasions. At three localities where the parasite was noted on a few cankers in 1926, no trace of it was found after a diligent search in 1931. During the intervening years it was

[4] The genus Ribes is used in this paper to include both currants and gooseberries.

rarely in evidence at these same localities. At Owl Creek, however, where experiments in the control of blister rust by the lilac fungus. The mode the parasite was noted as common in 1926 and very common in the following years, it may still be frequently found, yet its presence has not resulted in measurable reduction of blister-rust attack. The first damage by blister rust to western white pines at this place was also noted in 1926, when a few flags (dead branches) could be seen on these trees in an otherwise healthy-appearing stand of white pine and Douglas fir reproduction in approximately equal representation. Today the stand is almost pure Douglas fir, practically all the pines having been killed by the rust in spite of the abundance of the parasite. In this case, even with *T. maxima* sporulating abundantly each year, not all of the aecia were parasitized and aeciospore production was sufficient to cause abundant annual infection of Ribes with a resultant continued infection and killing of the pines. The other localities where the lilac fungus has been found are some of the oldest and most severe pine-infection centers in this region, and yet in these places the parasite has barely managed to perpetuate itself.

It is not uncommon to observe one tree of western white pine with numerous blister-rust infections, many of which are invaded by the parasite, while a few feet distant may be another similarly infected tree of about equal size on which no trace of the lilac fungus can be found. Also, on numerous occasions heavily infected pines have been observed with only one or two parasitized cankers.

This sporadic occurrence and behavior of *Tuberculina maxima* may indicate lack of virulence that, as compared with its more aggressive behavior as reported in Europe, suggests the possibility of different strains of the organism. If this be the case, it still remains to be seen just how readily the European fungus would attack blister rust in this country. Experiments and investigations in Europe (4, 10, 12, 13, 14, 15), however, have apparently not resulted in successful control of the rust with the aid of the parasite, for, in spite of its presence, the destruction of white pines by the rust in that country has continued. Perhaps it might be suggested that the blister rust in western North America is caused by a strain of *Cronartium ribicola* that is less susceptible to *T. maxima* than the common European strain. No positive indication, however, of the existence of physiological strains of blister rust has ever been found (10); hence, it is unlikely that this is a factor in the behavior of the lilac fungus. It is far more probable that its erratic behavior is caused by environmental conditions unfavorable to its propagation and spread.

Tuberculina maxima may offer some possibilities with respect to local control of the rust. Rohmeder (7) states that good results have been ob-

tained in several Swabian forest areas in controlling *Cronartium ribicola* by its natural enemy, *T. maxima*. Tubeuf (14) has reported successful of dissemination employed by the latter consisted of collecting the lilac-color spore dust of the parasite in a paper bag and transferring it to the aecia with the tip of a penknife. This procedure would, of course, be impracticable in extensive control operations such as are employed in this country.

Tubeuf (14) emphasizes that never are all white pines killed by the rust nor are all rust foci destroyed by *Tuberculina maxima* and that one may speak only of reduction, not of eradication. The writer knows of no instance of successful biological control of an active fungous parasite of plants. It appears, therefore, that little hope may be held for *T. maxima* as an important factor in the general control of *Cronartium ribicola* in this country.

SUMMARY

The lilac fungus (*Tuberculina maxima*), a parasite of the pycnia and aecia of several pine rusts, is reported for the first time in North America on *Cronartium ribicola* and on *C. comptoniae*.

The known distribution of the parasite on *C. ribicola* is limited to two small areas in British Columbia—one in the coastal and the other in the interior region—and to one locality in northeastern Washington. On *C. comptoniae* the parasite has been found only at Daisy Lake, B. C., in the coastal region.

There is no evidence that *T. maxima* was introduced into this country with the introduction of *C. ribicola* to western North America. There is evidence, however, that it is indigenous, probably on one or all of its native pitch-pine blister-rust hosts.

The parasite has never become generally destructive to either *C. ribicola* or *C. comptoniae*. It is not considered an important factor in reducing aecial sporulation or in controlling *C. ribicola*.

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ASPERGILLUS SCLEROTIORUM, N. SP., AND ITS RELATION TO DECAY OF APPLES[1]

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From 1926 to 1929 11 forms of Aspergillus were isolated by the writer from the surface of normal apples (5). Seven of these were found capable of causing decay on apples in common storage; none, however, caused decay in cold storage (0°–2° C.). Several species of Aspergillus have been isolated by other investigators (2, 3, 4, 7) from common storage and market apples, but none has been reported to cause decay in cold storage. Brooks and Cooley (1) found that apples inoculated with *Aspergillus niger* (Cram.) v. Tiegh., kept in the laboratory for 1 week after inoculation and then placed in cold storage, decayed. Apples, similarly treated and placed in cold storage immediately after inoculation, did not decay. In explanation of these results, they state that "all or nearly all of the rot was produced before the fruit was cooled."

Since 1929 3 additional forms of Aspergillus have been isolated by the writer from the surface of normal apples. Only one of these forms proved pathogenic on apples, causing decay at both common and cold-storage temperatures. When sound, ripe Jonathan apples were inoculated with mycelium and spores of this species and immediately placed in cold storage, a distinct decay was produced. This organism is presented here as a new species belonging to the *A. ochraceus* group, *sulphureus* series (9). The following description was made from cultures growing on Czapek's solution agar, as used by Thom and Church (9):

Aspergillus sclerotiorum, n. sp.

Colonies (Fig. 1, A) white at first, becoming sulphur yellow (Ridgway, Pl. V, 25) (8) with the maturing of the conidial heads and thickly seeded with light sclerotia; growth slow, reaching a diameter of 35 mm. in 10 days at 25° C.; reverse cream color. Conidial heads (Fig. 1, C, D) varying in the same colony from hemispherical to columnar, more or less loose, commonly splitting into 2 or more divergent columns, varying in size up to 140 μ wide with the hemispherical heads and up to 250 μ long by 140 μ wide with the columnar heads. Stalks light yellow, pitted, up to 1,200 μ long by 6 μ to 12 wide, slightly increasing in width toward the conidial heads. Vesicles globose to flask-shape, up to 40 μ in diameter. Sterig-

[1] Published as Scientific Paper No. 202, College of Agriculture and Experiment Station, State College of Washington.

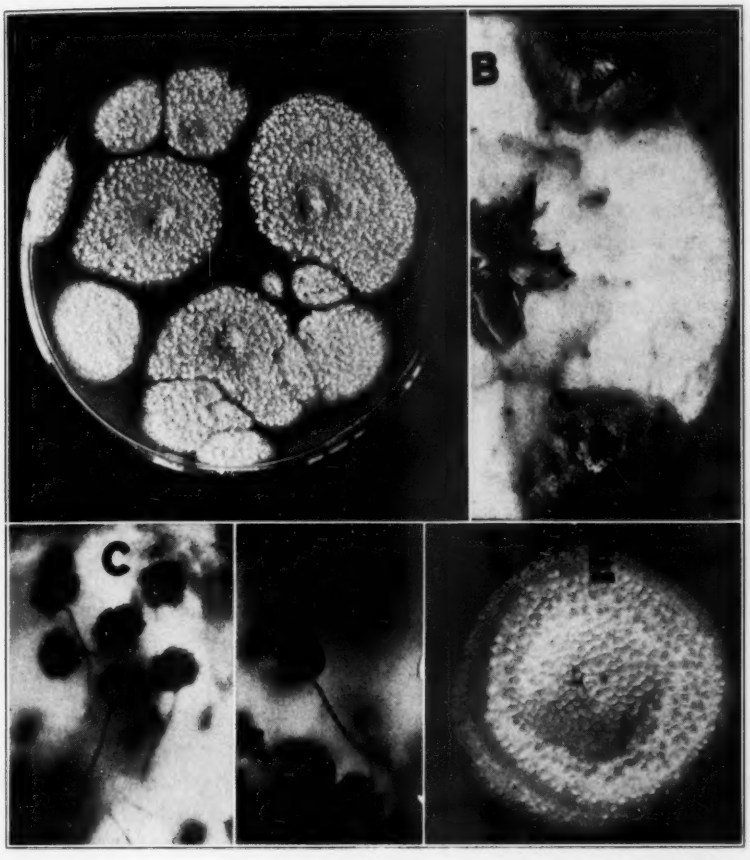

FIG. 1. *Aspergillus sclerotiorum.* A. Plate culture on Czapek's solution agar incubated at 25° C. for 12 days. B. Cross section of Jonathan apple, showing type of decay. C and D. Photomicrographs, showing hemispherical and columnar conidial heads, respectively. ×50. E. Carrot slice, showing abundant sclerotial development.

mata in 2 series, primary and secondary, approximately equal in length, up to 8.5 μ. Spores globose, smooth, 2 μ to 3 μ in diameter; walls slightly tinged with yellow. Perithecia not found. Sclerotia abundant, first appearing on cultures 3 days old, globose to subglobose, white at first, soon becoming light cream and then flesh pink (Ridgway, Pl. XIII, 5) (8), up to 1.5 mm. in diameter.

On sterile carrot slices,[2] mycelium white at first, turning pink with the beginning of sclerotial development. Conidial production sparse. Sclerotial development very abundant (Fig. 1, E).

[2] Sliced carrots placed in Petri dishes on wet filter paper and sterilized in an autoclave for 20 minutes at 15 pounds' pressure.

When inoculated (6) into sound, ripe Jonathan apples, the fungus produced lesions 32–36 mm. in diameter in 28 days at 22–25° C. In 42 days the lesions had attained diameters of 42–46 mm. Held in storage at 6–8° C., the fungus produced lesions 16–23 mm. in diameter in 42 days. At the end of 90 days the lesions had attained diameters of 28–38 mm. In cold storage (0–2° C.), it produced lesions 10–14 mm. in diameter in 120 days. The surfaces of the lesions were brown with a yellowish green tinge, slightly sunken, and tending toward concentric rings, which were very pronounced on the colored cheeks of the apples. The decayed tissue was dark tan, dry, and more or less spongy. The lesions generally had the form of flattened cones (Fig. 1, B), the diameter of the lesions exceeding the depth to which the decay penetrated.

SUMMARY

A new species of Aspergillus is described as belonging to the *A. ochraceus* group, *sulphureus* series. This organism, for which the name *A. sclerotiorum* is suggested, was found to be pathogenic on apples, causing decay both at ordinary and cold-storage temperatures. When inoculated into sound, ripe Jonathan apples it produced lesions 42–46 mm. in diameter in 42 days at 22–25° C. It produced lesions 28–38 mm. in diameter in 90 days at 6–8° C. and lesions 10–14 mm. in diameter in 120 days at 0–2° C.

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PHYTOPATHOLOGICAL NOTES

Bacterium cucurbitae on cucumber.—In describing the bacterial leaf spot of squash (*Bacterium cucurbitae*), the writer[1] made the statement that this disease had not been found on cucumbers and that inoculations on this plant produced pin-point infections that did not develop further. Prasad,[2] however, in 1931, reported finding in India a leaf spot of cucumber caused by an organism, which he proved to be pathogenic to cucumber and which he states is identical with *Bact. cucurbitae* in cultures. The spots are described as thin, brown, and water-soaked, and made conspicuous by a bright yellow halo.

The disease has now been found on cucumber in the United States, though doing only minor damage. (Fig. 1, A.) Early in September, 1931, O. C. Boyd[3] collected specimens in Bristol County, Massachusetts, which he forwarded to the writer with the following comment: "Earlier in the season I saw no typical angular leaf spot on cucumber in that county. This spot on rather old vines just about through bearing, does not appear much like angular spot, yet bacteria flow out profusely from sections." Isolations from five widely separated spots on these leaves produced only *Bacterium cucurbitae*. This organism was also isolated from Hubbard squash leaves from the same county; concerning its prevalence on squash, Boyd says:[3] "The latter leaf disease was to be found in every field I visited toward the end of the season and very severe in certain fields." It seems probable that here the disease spread from squash to cucumber. Inoculations have been produced on cucumber and squash with the isolations from the cucumber leaves, reisolations made, and infection again produced on cucumber. (Fig. 1, B.)

Lesions on cucumber may be round or somewhat angular. The thin, papery brown center has a reddish brown margin and may or may not be surrounded by a yellow halo. There is less tendency than in angular spot for the centers to tear out. The diseases are sufficiently similar, however, to make field identification difficult. MARY K. BRYAN, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[1] Bryan, Mary K. Bacterial leaf spot of squash. Jour. Agr. Res. 40: 385–391. 1930.

[2] Prasad, Hari Har. A note on bacterial leaf spot of Khira (*Cucumis sativus*). Indian Jour. Sci. 1: 289–290. 1931.

[3] By letter.

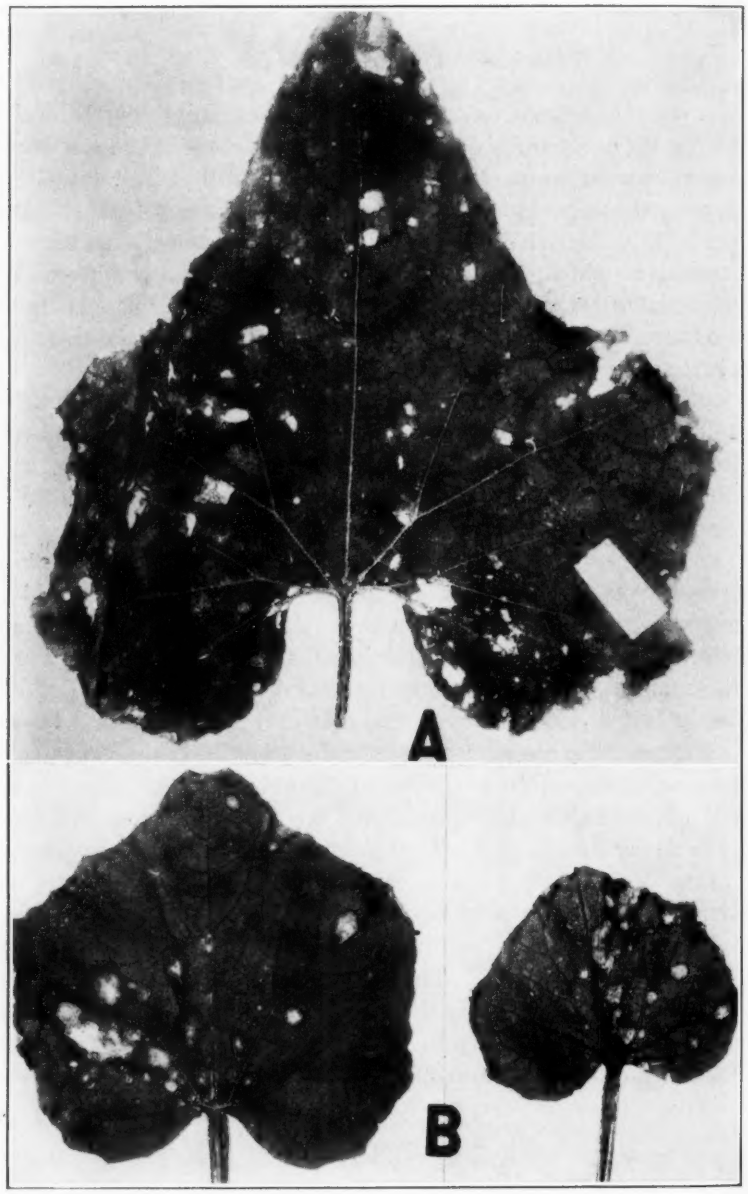

FIG. 1. A. Cucumber leaf collected in Massachusetts from which *Bacterium cucurbitae* was isolated. B. Cucumber leaves from a young plant sprayed with the isolation from leaf shown in A.

Cucumber Mosaic on Tobacco in Wisconsin.—Natural field infection of tobacco with the cucumber-mosaic virus has previously been known to occur in small percentages in different States. In 1932, field observations of mosaic symptoms on tobacco in Wisconsin suggested the widespread occurrence of the cucumber-mosaic virus on this host. In order to verify this observation, samples from 60 tobacco plants, representing 25 fields in various parts of the State, were selected and tested by inoculation to young greenhouse plants. Twenty-six plants yielded typical cucumber-mosaic virus; 21 yielded ordinary tobacco-mosaic virus; and in 13 cases the specific virus was apparently neither of the above but was not definitely identified. Plants that yielded ordinary tobacco-mosaic virus may in some instances have contained cucumber-mosaic virus also, since no separation of possible combinations was attempted. In one station field at Madison, about 30 per cent of the plants were affected with cucumber mosaic and a high percentage with ordinary tobacco mosaic. Judging from the symptoms manifested, cucumber mosaic was present in still higher percentages in parts of other fields in the State. More limited observations and inoculations indicated a similar situation with respect to mosaic on tomato. It was evident that cucumber mosaic was occurring in epidemic form on tobacco in 1932 in Wisconsin. On the basis of field observations in past years, it is believed that the occurrence of this disease on tobacco in epidemic form is unusual. The mode of transmission in this particular epidemic was not determined. Should cucumber mosaic occur regularly on tobacco in association with ordinary tobacco mosaic, control measures will naturally be complicated.—JAMES JOHNSON, Wisconsin Agricultural Experiment Station and U. S. Department of Agriculture.

BOOK REVIEWS

Insects and Diseases of Ornamental Trees and Shrubs. By E. P. Felt and W. H. Rankin. xix + 507 pp., 243 figs. The Macmillan Co., New York. 1932.

In this volume the authors have presented a combined revision of their former books, "Manual of Tree and Shrub Insects", by E. P. Felt, and "Manual of Tree Diseases", by W. H. Rankin. Some portions have been rewritten with additions of more up-to-date information, but much of the subject matter is simply a reorganization of the previous books. The volume is one of "The Rural Science Series" and is therefore of a semi-popular nature. Since plant pathologists will be interested particularly in the presentation of the diseases, this review will be concerned chiefly with that phase of the volume.

The book is divided into 2 parts, the first designated as "General: insects, fungi, injuries and treatments". There are 4 chapters in this section, covering 131 pages. The first chapter, of some 38 pages, deals with "Insects and their depredations". The second chapter, conforming with this first heading, is entitled "Fungi and their depredations". The 60 pages of the chapter are devoted to brief discussions of the following subjects—How the organism causes diseases; Diseases common to all trees—including leaf spots, powdery mildews, sooty molds, leaf cast of conifers, slime flux, lichen injury, mistletoe parasites, and the control measures in each case; Wood decay and root diseases, including heartwood decays, wound decay, basal decay and root diseases; Wounds and cavities in deciduous trees with treatments. Although much of this material was contained in the earlier "Manual of Tree Diseases", the concise description of the manner in which infection takes place and the relation of factors of environment will be a welcome addition for the general reader. The last subject in the chapter—wounds and cavities with treatments—is new material and takes the place of the chapter on "Tree Surgery" in the earlier "Manual". However, the author does not recommend that the tree owner undertake the treatment of wounds but emphasizes his belief that "Such work, as well as the more intricate methods of treating cavities, must be done by those with experience, the proper tools and accredited training in definite methods." He states briefly the principles underlying wound treatment and outlines the various processes of cavity treatment used in the past. All discussion of specific materials and equipment for cavity filling is omitted, and the reader is not referred elsewhere for such information.

Chapter III, entitled "Insecticides and Fungicides", describes Bordeaux, lime-sulphur and sulphur dust or spray. In the 3 paragraphs devoted to "Spraying Apparatus" the qualifications of a satisfactory apparatus are given, but undue emphasis has been placed on the difficulties in-

volved in the process of spraying, so that tree owners or gardeners would hesitate to undertake it themselves. In fact, the authors state that it requires expert knowledge to determine the proper time for spraying and that only well-qualified men should be intrusted with such work. It is evident that the book is written from the standpoint of the commercial arborist, who is not interested in explaining to amateur gardeners how to carry out the control measures themselves.

"Injuries other than those caused by organisms" are described in the 20 pages of Chapter IV. The first three headings—Root injuries, Body and branch injuries, and Leaf injuries—cover general discussions of the relation of environmental factors to the health of the various parts of the tree. These are followed by the descriptions of specific injuries in much the same manner as in the "Manual" with headings:—Injuries due to water relations; Freezing injuries; Gas and smoke injuries; and Electrical injuries.

Part II is devoted to an account of specific insects and diseases that attack ornamental trees and shrubs. The arrangement is according to the alphabetical listing of about 70 generic hosts, each genus being designated by its common English name. In a few cases, the separation of species of a generic host is made when the common names of the species differ, for example, azalea and rhododendron, buckeye and horse chestnut, cherry and plum. About 40 of the hosts are additions to the list in the "Manual" and include ornamental fruit trees and shrubs. Insect pests are described for all of these new hosts, but diseases are mentioned for only half of them. For many hosts the discussion of diseases and insects conforms to a definite arrangement with paragraph headings:—Insects attacking leaves; Leaf diseases; Insects attacking twigs and bark; Diseases of twigs and bark; Insects of trunk and wood; Diseases of trunk, wood and roots. Unfortunately, this arrangement cannot be consistently maintained, since for some of the hosts very few diseases have ever been reported. In a few cases, however, there is no apparent reason for the digression from the general scheme. Usually, each disease is described in a concise paragraph, including distribution, causal fungus, symptoms, and control. In most cases, the first word or words of a paragraph indicate the common name of the disease to be described. Here, again, there is frequently a digression from this scheme, sufficient to cause confusion in locating readily a particular disease. The less important diseases are sometimes grouped in one paragraph with a few sentences devoted to each disease. Unless one knows the name of the causal organism, it is difficult to locate the description of any particular disease. For example, 5 apple cankers are described in 1 paragraph covering 2 pages with only the fungus names in italics to distinguish them readily. A very complete index, however, tends to counteract the difficulties of the arrangement.

The book, as the authors state in the preface, "does not represent the final word," but "is a summation of available information to date." On the whole, a careful selection of the most common diseases has been made, including those that have not yet been satisfactorily studied but that have already given indication of their importance. In the cases of some of the shrubs, however, brief mention of a few other common diseases might have been included, such as the mildew of crape myrtle, leaf spots of holly and of English ivy. The omission of such mention gives an erroneous impression that no diseases have been reported on these hosts.

The most regrettable change from the earlier "Manual" is the complete elimination of a bibliography. This seems particularly unfortunate in connection with those diseases that have been recently reported or in which the causal organisms have not yet been discovered. Undoubtedly, the value of the book for readers familiar with fungi and for students of plant pathology is considerably lessened by this omission, since additional information will be desired to supplement the brief descriptions and to clarify vague statements. The book is intended primarily for those unacquainted with the technical aspects of tree diseases but interested only in finding out what is the cause of certain symptoms of disease. For this purpose the information is adequate and the book is an acceptable addition to the "Rural Science Series".—ALMA M. WATERMAN, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with Brown University, Providence, R. I.

Shear, C. L., Neil E. Stevens, and Henry F. Bain. Fungous Diseases of the Cultivated Cranberry. U. S. Dept. Agr. Tech. Bul. 258: 1–56 pp. 4 pl. (2 col. pl.), 35 fig. 1931.

To consider in the category of a book a bulletin, regardless of the source, may seem somewhat out of place; yet, when that bulletin is a plant-disease monograph, it changes from a run-of-the-mill publication of scientific research and becomes a reference work. Hence, the publication by the United States Department of Agriculture of "Fungous Diseases of the Cultivated Cranberry," by C. L. Shear, Neil E. Stevens, and Henry F. Bain, is of especial moment.

About 3 years ago, it was my pleasure and privilege to chronicle the advent of Harter and Weimer's "A Monographic Study of the Diseases of Sweet Potatoes and their Control," the first of such publications to be issued by any organization. Now, it is my good fortune to call the attention of plant pathologists and mycologists to a similar monograph, similar, however, in that it is a monographic treatment of diseases. Where Harter and Weimer devoted their space principally to the several diseases as conditions that demand prophylactic and other treatment, the present authors consider diseases more in the light of their causal organisms. In fact, 39

of the few more than 50 pages of text are devoted to the taxonomy and physiology of the rot-producing fungi.

Whether the discussion is of important rot fungi, fungi causing diseases of cranberry vines, or cranberry fungi of minor importance, the general treatment, taxonomically, is the same, for an example of which the treatment of storage-rotter *Diaporthe vaccinii* may be cited: Synonymy, pycnidia, perithecia, type specimen, cultural characters on artificial media, hosts, geographical distribution, genetic relationship between Phomopsis and Diaporthe, pathological relations.

Into the physiology of the rot fungi the authors have delved at some length, taking up in order time of infection, dissemination by water, aridity relations, temperature relations, relative abundance of cranberry fungi in different regions (with considerations of the field rots, relative importance of different fungi as the cause of storage rots, and succession of fungi in storage), climates of different cranberry sections in relation to the abundance of various fungi (including a discussion of the factors bearing on sterile breakdown), and the relation between growing-season weather and keeping quality of the cranberry crop in Massachusetts.

The control of the fungous diseases of cranberries is discussed under the headings of vine diseases and cranberry fruit rots. In the former case, individual diseases—fairy ring, red gall, rose bloom, red leaf spot, black spot, hard rot (cotton ball) and tip blight—are briefly treated, as they "are field diseases and may fairly be classed as minor troubles."

With fruit rots, however, it is another story, and the authors go into some detail regarding the control of these diseases, first pointing out the differences in climate, cultural conditions, varieties, and marketing policy in the several cranberry-growing regions, which are of significance in control measures. As to actual measures for control, spraying with Bordeaux 4-4-½-50 (the ½ being commercial fish-oil soap) is but one of 6 headings under which control measures are advocated, the other 5 being bog management, harvesting methods, handling methods, storage, and marketing the crop.

Synonyms for names of host plants, a "Literature cited" of 86 titles, and an index to fungi are given.

Small in size, this cranberry-disease monograph is great in its make-up and usefulness. Any one who has had occasion to search the literature on any subject whatever well realizes the flagrant lack of adequate compedia. In sponsoring the sweet-potato- and cranberry-disease monographs, the United States Department of Agriculture has performed a gracious act for which pathologists, regardless of where they may be, should be duly thankful.

No less appreciative of Messrs. Shear, Stevens, and Bain should be their fellow workers, for they have issued a work that may well serve as a model for later efforts.—W. A. WHITNEY, Washington, D. C.

Acta Phaenologica, an international bimonthly journal of phenology sponsored by the *Nederlandsche Phaenologische Vereeniging*, and published by Martinus Nijhoff, The Hague. Beginning with the year 1932.

Adding another outlet of publication to the already oversupplied field of natural science is a task that may be approached with fear and trembling. However, there are branches of research that up to the present time are without an adequate organ for the dissemination of their results: a journal that will be particularly adapted for and devoted primarily to a restricted field. Accordingly, *Acta Phaenologica* is welcomed into the family of scientific publications.

Sponsored by the *Nederlandsche Phaenologische Vereeniging* under the secretarial supervision of Dr. H. Bos of that institution, the *Acta* appears as a bimonthly record of phenological research, open to any worker whether or not affiliated with the Dutch society.

In offering the initial number, the editor outlines his policy:

> The periodical will welcome articles of different character and substance. Several will be original works . . . others less detailed. . . . We will insert also summaries of articles which have appeared elsewhere, either in other periodicals or in books. . . . We shall attempt to give also bibliographical bulletins, including the appearing of annual phenological reports. . . . Moreover, we intend to announce problems which call for general collaboration, observation, methods of operating, and notation. We shall welcome questions and answers, "personalia," desiderata, offers regarding the borrowing and exchanging of objects, manuscripts, books, statistics, etc., and finally suggestions, concerning the form, editorship, and organization proper, on condition that discussion of these last points be deferred until after completion of the first volume.

Naturally with so wide a scope and purpose, the happy culmination of the sponsor's dream is more than ordinarily dependent upon the wholehearted cooperation of all interested parties. So worthy a publication should surely receive support, and I, in the editor's language, heartily "urge everyone who is convinced of the necessity of a consolidation of phenological material to subscribe immediately, so that the publisher, at the end of the trial-period of three years, need not be obliged to discontinue the publication, and we express the hope that at the end of this period the new journal may be firmly established."

The first number of the *Acta Phaenologica* is one of 40 pages, of which 9 and a fraction are taken by the editor's introduction, with French and German renditions of the English. Dr. H. Bos outlines the "*Begriff und Zukunft der Phaenologie*" (pp. 11–22). "The Cold Spring of 1929 in the British Isles. I. Reaction by Insects and Birds," by J. Edmund Clarke, comprises pages 23 to 28. S. Illichevsky of the Union of Soviet Socialist Republics describes "The Results of the Phenological Observations at

Poltava" (p. 29–37). In a short note J. C. Pfeiffer reports on "*Phaenologie et Mauvaises Herbes*" (p. 38). The last 2 pages (39 and 40) are taken up with an author abstract of E. Hiltner's "*Die Phänologie und ihre Bedeutung für die Klimatische Behandlung der Pollenallergie*" in Gutman's "*Die Pollenallergie; Heuschnupfen, Heufieber, Heuasthma.*"

In format the *Acta Phaenologica* presents a creditable appearance, its type-page, $6\frac{3}{4} \times 4\frac{1}{8}$ inches, a clear type face, and a not-too-glossy paper combining to make a page very easily read. Though without half-tones or cuts of any kind, there seems to be no reason why the illustrations of later numbers should not be well reproduced, of course, with the natural proviso that the originals are worthy.

Of particular interest and importance are the language requirements—or lack of them—in the journal. Articles are accepted when written in English, French, or German, provided summaries in the other two languages also are submitted. This requirement might well be copied in other journals of "international" character. Such summaries will add immensely to the use of the journal in every land, offering to the unilingual scientist (and, in spite of language requirements in advanced university training, there are many of them!) a wider acquaintance with the literature.

Whether the *Acta Phaenologica* fills an essential gap in scientific periodicals or whether the papers within the scope of the journal might as fittingly—if not actually more so—be printed in a publication more closely allied to the subject whose climatic relations are discussed is a moot question, the definite answer to which must of necessity be determined by the individual author as each case arises. For example, a paper treating of the epidemiology of *Colletotrichum lindemuthianum* might better be issued in PHYTOPATHOLOGY or some other phytopathological journal, thus reaching an audience having somewhat more than an academic interest in the phenological phenomena presented.

On the other hand, however, there are papers whose theme and treatment are phenological first, with other considerations coming later. For such papers there has been no logical place in which to publish. Because of this, it is not to be inferred by the previous statement that the *Acta Phaenologica* is superfluous: it most decidedly is not, and, complementing other journals, it closes the hiatus in the literature sources between natural sciences *per se*, on the one hand, and pure meteorology, on the other.

In recent years the consideration of the relation of weather to the occurrence of plant phenomena in general and diseases in particular has come increasingly to the fore, yet, despite these advances, especially in the epidemiology of cereal rusts that have been made, the potentialities of the field are as yet unrealized. The appearance of the *Acta Phaenologica* can not fail to further the advances in its line by furnishing not only a logical but a highly commendable place for publication.—W. A. WHITNEY, Washington, D. C.